Inorganica Chimica Acta, *107* (1985) 247–252

The Binding of Metal Ions by Captopril (SQ 14225). Part I. Complexation of Zinc(II), Cadmium(II) and Lead(II)

M. A. HUGHES, G. L. SMITH and D. R. WILLIAMS*

Department of Applied Chemistry, UWIST, P.O. Box 13, Cardiff CF1 3XF, U.K.

Received February 13, 1985

Abstract

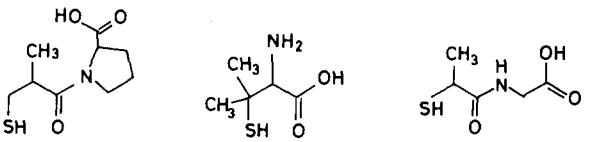
A number of side effects have been observed in the use of captopril therapy. Many of these symptoms are typical of trace element deficiency and it seems probable that this agent complexes metal ions *in vivo*. Investigations into the interactions of captopril with Zn(II), Cd(II) and Pb(II) ions are carried out using glass electrode potentiometry under conditions of 37 °C, 150 mmol dm^{-3} NaCl. The formation constant data are then used in computer simulation models to investigate the mobilisation of these metal ions by captopril in normal plasma. It is shown that mobilisation is unlikely at pharmacological levels of the drugs, and therefore side effects are probably not directly dependent on mobilisation of these metals.

Introduction

It has been known for many years that the venoms from several species of snake have an effect on blood pressure, in most cases reducing it. In the late 1960s, researchers were successful in fractionating the vasodilating components of the venom of the South American snake, *Bothrops Jararaca* [1]. The most active component was a nonapeptide, teprotide, SQ 20881, which proved to be very effective in the treatment of hypertension in man [2]. However, teprotide had the disadvantages of beinb costly to synthesise and not orally active.

Work on determining which fragments of the nonapeptide were important for activity, and trying out many modifications eventually led to the development of captopril, 1-[2(S)-3-mercapto-2-methylpropionyl]-L-proline, SQ 14225, first synthesised in 1975 [3–6] (Scheme 1).

Captopril can be economically produced, is orally active, and is an effective hypotensive agent, reducing blood pressure in many patients whose hypertension



Captopril Penicillamine Tiopronin

Scheme 1.

could not be controlled by other available drugs [7–10]. It has also been successful in the treatment of chronic congestive heart failure including many cases resistant to conventional therapy [11–13], and has led to dramatic reversal of vascular and renal crises of scleroderma [14]. More recently captopril has been used with some success to treat rheumatoid arthritis [15] and migraine [16].

Captopril may succeed in lowering blood pressure in cases where other drugs have failed because, like teprotide, its mode of action is different from that of earlier antihypertensive agents. Captopril was specifically designed to inhibit the enzyme that converts angiotensin I to angiotensin II, a potent vasoconstrictor. The plasma levels of the latter are often elevated in hypertensives, and inhibition of its formation is one of the principal ways in which captopril reduces blood pressure [6, 8].

Side Effects

As clinical trials and subsequent use of captopril progressed, it became evident that there were side effects associated with the larger doses of drug (>450 mg day^{-1}) [17, 8]. The dose required for treating patients with congestive heart failure (C.H.F.) was considerably lower and the frequency of side effects was half that among hypertensives receiving captopril [13]. It was later found that reduction of the dose to 150 mg or less per day often allowed adequate blood pressure control [18, 19]. These lower doses diminished the frequency of side effects from 10% to 5% for rash, and from 7% to 4% for dysgeusia [20]. Neutropenia, the most serious, though not the most frequent, adverse effect occurred in 0.04% of patients on the higher dose. These

*Author to whom correspondence should be addressed.

0020-1693/85/$3.30

clinical effects were particularly prevalent in patients with both impaired renal function and collagen vascular disease (C.V.D.) where the frequency was 7.2% [21]. Even in these patients the occurrence of neutropenia was reduced to 0.3% on the low dose of 150 mg (max) per day. Among a group of 1720 patients with neither renal disease nor C.V.D. who were kept on low dose there were no cases of neutropenia. As captopril is excreted exclusively via the kidneys it is possible that renal impairment leads to some accumulation of the drug [22].

The side effects of early therapy were similar to those reported for D-penicillamine [23–25] and other thiols such as thiopyridoxine and tiopronin (alpha-mercaptopropionylglycine, MPG) [26, 27], although the incidence of side effects was considerably higher with penicillamine [23, 25, 28] than with captopril. There are distinct structural similarities between captopril, penicillamine and tiopronin, all having basically similar complexing groups, including a sulphydryl group, though their amine groups are tertiary, primary and secondary, respectively.

It has been suggested that interaction of these drugs with trace metal ions might be involved in the production of side effects, particularly as zinc deficiency has been shown to cause taste dysfunction [29–35]. Copper depletion has also been considered [24, 28], and Knudson and Weismann suggested that involvement of copper is more certain than that of zinc when taste dysfunction is induced by penicillamine therapy [24]. Clinical studies have shown that only 4% of patients with Wilson's disease exhibited hypogeusia following D-penicillamine treatment, in contrast to an incidence of 32% in patients receiving the drug for other diseases [28]. Some studies have suggested that hypogeusia may be related to an elevated level of thiols with no demonstrable change in metal levels in the body [36]. Of the other side effects neutropenia has been associated with copper deficiency [37] and skin rash with zinc depletion [38, 39].

As in the case of penicillamine [40], it has been suggested that the nature and time course of adverse reactions to captopril are consistent with dysregulation of the immune system, the drug acting either as a hapten or by pharmacological modulation [41, 42]. Other researchers consider that a pharmacological mechanism is more likely than an immunological one, particularly as adverse reactions often resolve at lower dosage schedules [43, 44]. An immunological mechanism would not necessarily exclude the involvement of metal ions, several thiols acting with copper, for example, being known to reduce T cell function *in vitro* [45–47]. There is also evidence for zinc involvement in the integrity of the immune system [38].

As captopril is known to interact with zinc in the active site of angiotensin converting enzyme (ACE) [5, 65], it was decided to determine the metal binding properties of captopril *in vitro* by potentiometry, and to predict them *in vivo* in normal blood plasma by computer simulation.

Experimental

Materials

Analytical grade reagents were used throughout. All solutions were prepared using distilled, doubly deionised, degassed water. Solutions of captopril were freshly prepared each day.

Reagents

Solutions were prepared by direct weighing. Captopril, (Squibb), was used without further purification. (*Anal.* Found: C, 49.9; H, 7.0; N, 6.5. Calcd. for $C_9H_{15}O_3NS$: C, 49.7; H, 6.9; N, 6.5%). Stock solutions of metal ions were prepared from their chloride salt and determined volumetrically against EDTA [48]. Their mineral acid content was determined by titration with standard alkali and the concentration optimised by use of the MAGEC program [49]. Sodium hydroxide solutions (100 mmol dm^{-3}) and hydrochloric acid solutions were prepared from ampoules (BDH concentrated volumetric solutions).

Methods

Potentiometric titrations were performed as described in ref. [50] except that sodium chloride 150 mmol dm^{-3} was used as the background electrolyte instead of sodium perchlorate. Total ligand concentrations generally ranged from 3 to 15 mmol dm^{-3}.

The estimated protonation and metal ligand formation constants were optimised using the MINIQUAD program [51] and the parameters for ligand protonation titrations, generally E_{const}, were optimised using the MAGEC program. For the ligand protonation and metal ligand systems, curves were plotted using the ZPLOT program [52, 53] and ESTA* program [54]. The search for species, and optimisation of their formation constants were carried out using MINIQUAD. *In vitro* species distribution curves were generated using these constants in ESTA. The plasma mobilisation of metal ions by captopril was simulated using the ECCLES** program [55]. The program calculates a Plasma Mobilising Index (PMI) for each metal ion, and for a range of drug concentrations from 10^{-9} to 10^{-3} mol dm^{-3}.

*Equilibrium Simulation for Titration Analysis.

**Evaluation of Constituent Concentrations in Large Equilibrium Systems.

TABLE I. Thermodynamic Formation Constants for Captopril. 37.0 °C and 150 mmol dm^{-3} Sodium Chloride ($\beta_{pqr} = [L_pM_qH_r]/[L]^p[M]^q[H]^r$.

Species p	q	r	lg β (sd)	Sum of squared residuals	R factor	Number of points	Number of titrations
Protonation							
1	0	1	9.681(0.001)	2.83×10^{-6}	0.002	285	6
1	0	2	13.150(0.002)				
Zinc							
1	1	0	5.38(0.03)	2.03×10^{-6}	0.004	401	7
2	1	0	11.66(0.01)				
3	1	0	15.30(0.03)				
1	1	−1	−2.14(0.11)				
2	1	−1	1.65(0.05)				
Cadmium							
1	1	0	6.26(0.08)	2.25×10^{-6}	0.006	377	9
2	1	0	13.13(0.02)				
3	1	0	17.47(0.03)				
3	2	0	23.33(0.02)				
4	4	0	35.90(0.03)				
2	1	−1	3.45(0.11)				
Lead							
1	1	0	9.53(0.10)	4.58×10^{-7}	0.003	316	8
3	1	0	16.49(0.02)				
3	2	0	23.73(0.01)				
4	2	0	28.58(0.03)				
3	1	−1	5.40(0.08)				

$$\text{PMI} = \frac{\text{total concentration of low molecular weight metal complexes in presence of drug}}{\text{total concentration of low molecular weight metal complexes in normal blood plasma}}$$

Results and Discussion

Formation Constants

The thermodynamic formation constants for the protonation of captopril and for its interactions with Zn(II), Cd(II) and Pb(II) ions are shown in Table I. There was no significant binding of captopril to magnesium, calcium, manganese or iron(II).

In the captopril–zinc system, only mononuclear species were found, the most stable complex being the ML_2 which complexes > 50% of zinc from pH 6 to 8.2 at ligand to metal ratios greater than 3:1.

In the captopril–cadmium system the formation curve is a function of the metal concentration, as well as of the ligand concentration, in the lower pH range from pH 3.5 to 6.0. This indicates the presence of complexes containing more than one metal ion. It is known that ligands containing RS- groups readily form metal complexes in which sulphur bridges link two or more metal ions [56, 57]. At a ligand to metal ratio of 14.7:7.0 mmol dm^{-3} the M_4L_4 complex accounts for 44% of metal complexes at pH 5.0. The M_2L_3 species then becomes predominant, complexing 67% of the metal at pH 6.0. Above pH 7.0 the formation curves become superimposable indicating that mononuclear species are the predominating complexes in this region.

Polynuclear species were found in the captopril–lead system, although it was only possible to vary metal concentrations from 1.3 to 3.4 mmol dm^{-3}, due to the low solubility of the lead chloride (stock solution 5 mmol dm^{-3}). A point of inflexion in the metal–ligand formation curve at Zbar = 1.5 indicates a stable M_2L_3 type of polynuclear species. Analysis of data showed M_2L_3 formation accounts for 70% of the metal complexed at pH 6, with the M_2L_4 accounting for up to 70% at pH 8. The mononuclear species ML complexed 46% of the metal at pH 5 and the ML_3 accounted for up to 87% at pH > 9.

Titrations were also carried out with the captopril–nickel system but drifting of electrode poten-

tial in the pH range 6.5 to 8.0 was a problem. This may have been due to the system being slow to reach equilibrium in this region.

In Vivo

Captopril is rapidly absorbed from the gastrointestinal tract, (about 70% or the oral dose being absorbed in fasting subjects), and soon becomes about 30% bound to plasma proteins [41, 58]. Pharmacokinetic studies on captopril have considered single oral formulation in normotensive patients [59], low doses to congestive heart failure patients [60], and acute and chronic administration to hypertensive patients [61, 62]. Peak plasma levels of captopril are three times higher in patients who have been on captopril for some time than after single dosage [63], and the highest peak plasma level reported is 2×10^{-5} mol dm^{-3} [61]. Captopril is usually rapidly metabolised so such levels are only transient, the concentration of unchanged captopril falling quickly towards zero within three hours.

At a concentration of 10^{-5} mol dm^{-3} the ECCLES simulation shows only 0.4% of zinc bound. This would rise to 33.2% at a captopril concentration of 10^{-3} mol dm^{-3}, 23% of zinc being bound in a ternary complex $CapCysZn^{2-}$, and smaller fractions in a ternary complex with histidine and in the captopril zinc bis species. However such a concentration of captopril is much higher than would normally be found *in vivo*. The PMI curves for the mobilisation of zinc and cadmium by captopril are shown in Fig. 1, and the computed distributions of captopril species in Table II. The PMI curve of penicillamine for zinc is shown for comparison.

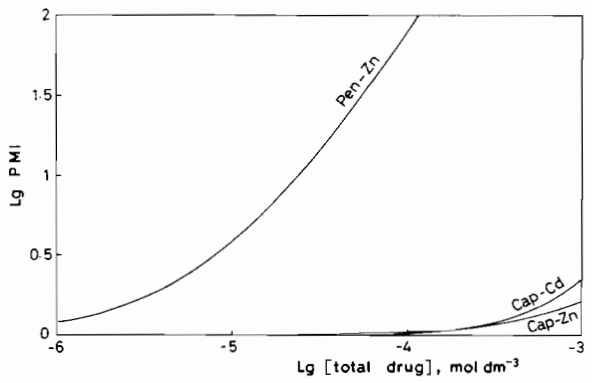


Fig. 1. PMI curves for the mobilisation of zinc and cadmium by captopril, compared with that of zinc by penicillamine. For abbreviations see footnote [a] Table II.

The PMI for lead is zero even at the highest scanned concentration of 10^{-3} mol dm^{-3} captopril.

The peak ratio of total captopril to unchanged captopril is generally 2:1 [59]. However, in renal failure hypertensive patients [62] and in CHF patients [60] it can be as high as 6:1. This suggests accumulation of the drug, and a study to determine the kinetic parameters of captopril indicated extensive partitioning into the deep tissues [64].

TABLE II. Computed Low Molecular Weight Complexes of Captopril in Human Blood Plasma.[a]

Species	% Metal bound		
	Drug concentration (mol dm^{-3})		
	10^{-5}	10^{-4}	10^{-3}
Zinc(II)			
$ZnCapCys^{2-}$	0.4	3.6	23.4
$ZnCapHis^{-}$	0.0	0.8	5.1
$ZnCap_2^{2-}$	0.0	0.0	4.7
Cadmium(II)			
$CdCap_2^{2-}$	0.0	0.0	33.6
$CdCapCys^{2-}$	0.3	2.5	11.9
$CdCapCysH^{-}$	0.1	1.0	4.8

[a]Abbreviations: Cap = Captopril, Cys = Cysteine, Pen = Penicillamine, His = Histidine.

It is suggested that the disulphide metabolites (mixed disulphides of cysteine, glutathione, albumin and a small amount of captopril disulphide) may act as a reservoir of pharmacologically active drug. This would explain in part why the half life of pharmacological activity is greater than the apparent half life of captopril in blood [63]. *In vitro* studies on the biotransformation of captopril disulphide showed that it was transformed almost exclusively to free captopril in the rat liver, and extensively so in the lung preparations, but mainly to mixed disulphides in the kidney [64]. A study on the disposition of captopril in rats [67] indicated that half lives for total captopril in tissues decreased in the order:

skin > other tissues > plasma

Metal Supplementation

Because of the possible involvement of metal–drug interactions in side effects, metal supplementation has been considered. Some of the available reports on oral administration of zinc or copper in cases of thiol-related hypogeusia seem to be inconsistent with regard to their effect on taste function, and the number of cases where details are to hand are comparatively few [28, 68, 69, 42]. In one case, four days of nickel supplementation effected a cure of hypogeusia, in another case zinc supplementation was effective, but only after it had been given for two months [26]. Henkin now considers that no more than 25% of patients with taste and smell

dysfunction would be expected to exhibit zinc deficiency [39]. Jaffe gave daily copper supplement to all patients on commencing penicillamine therapy in an attempt to prevent renal lesion and/or impaired taste function from developing [70]. Two years later he reported that data were still insufficient to permit a conclusion [71]. High dose copper supplementation has also brought about reversal of gross changes of skin, bone and cartilage to normal in penicillamine-treated rats [72].

There is comparatively little reported on measurement of trace metal levels in, or metal supplementation for, patients on Captopril. McNeil had three of sixteen patients develop taste loss, two of whom received zinc supplements without apparent effect [73] but no indication was given of the dosage or duration of the zinc supplementation. MacGregor *et al.* reported taste loss in six out of forty seven patients, but they have been unable to show any change in plasma zinc or copper levels with captopril, and did not report any attempt at copper or zinc supplementation [74].

Smit *et al.* reported one case of a sixty-year old man with polycystic kidney disease who suffered ageusia, skin rash and baldness on captopril treatment although serum zinc was in the normal range. The conditions were almost completely reversed after three months of zinc supplementation [75].

Captopril–Copper Interactions

Formation constants for captopril–copper(II) interactions could not be obtained from a study of the binary system, solutions at pH < 4 yielding a precipitate, while at pH > 4 the formation curves were not superimposable. This was probably because redoxing was taking place as is common in copper(II)–thiol reactions [76, 77].

It has been suggested that the redoxing process in such systems could be reversed or inhibited to some extent in the presence of imidazole or peptide ligands [78], and several studies have been made using this concept [79, 80, 81]. It was therefore decided to use this approach to investigate the captopril–copper interactions. These investigations are currently under way and will be reported in a subsequent communication. Preliminary analysis indicated that captopril could possibly mobilise copper in plasma, and *in vivo* studies on urinary excretion of copper and zinc in hypertensive patients being treated with captopril are to be undertaken.

Acknowledgement

We wish to thank E. R. Squibb for generously supplying us with samples of captopril.

References

1 S. H. Ferreira, D. C. Bartelt and L. J. Greene, *Biochemistry, 9,* 2583 (1970).
2 H. Gavras, H. R. Brunner, J. H. Laragh, I. Gavras and R. A. Vukovich, *Clin. Sci. Mol. Med., 48,* 57s (1975).
3 D. W. Cushman, J. Pluscec, N. J. Williams, E. R. Weaver, E. F. Sabo, O, Kocy, H. S. Cheung and M. A. Ondetti, *Experientia, 29,* 1032 (1973).
4 M. A. Ondetti, B. Rubin and D. W. Cushman, *Science, 196,* 441 (1977).
5 P. A. Diassi, in F. Gross and R. K. Leidtke (eds.), 'Pharmacology and Clinical Use of Angiotensin I Converting Enzyme Inhibitors', Gustav Fischer Verlag, Stuttgart/New York, 1980.
6 D. W. Cushman, H. S. Cheung, E. F. Sabo and M. A. Ondetti, in Z. P. Horovitz (ed.), 'Angiotensin-Converting Enzyme Inhibitors', Urban and Schwarzenberg, Munich, 1981.
7 H. R. Brunner, H. Gavras, B. Waeber, G. R. Kershaw, G. A. Turini, R. A. Vukovich, D. N. McKinstry and I. Gavras, *Ann. Intern. Med., 90,* 19 (1979).
8 R. C. Heel, R. N. Brogden, T. M. Speight and G. S. Avery, *Drugs, 20,* 409 (1980).
9 H. R. Brunner, H. Gavras, B. Waeber, S. C. Textor, G. A. Turini and J. P. Wauters, *Hypertension, 2,* 558 (1980).
10 D. B. Case, S. A. Atlas, P. A. Sullivan, and J. H. Laragh, *Circulation, 64,* 765 (1981).
11 R. Davis, H. S. Ribner, E. Keung, E. H. Sonnenblick and T. H. Lejemtel, *N. Engl. J. Med., 301,* 117 (1979).
12 D. N. Sharpe, R. J. Coxon, J. E. Douglas and B. Long, *Lancet, II,* 1154 (1980).
13 J. A. Romankiewicz, R. N. Brogden, R. C. Heel, T. M. Speight and G. S. Avery, *Drugs, 25,* 6 (1983).
14 J. A. Lopez-Ovejero, S. D. Saal, W. A. D'Angelo, J. S. Cheigh, K. H. Stenzel and J. H. Laragh, *N. Engl., J. Med., 300,* 1417 (1979).
15 M. F. R. Martin, F. McKenna, H. A. Bird, K. E. Surrall, J. S. Dixon and V. Wright, *Lancet, I,* 1325 (1984).
16 F. Sicuteri, *Adv. Exp. Med. Biol., 156B,* 1141 (1983).
17 *Lancet, II,* 129 (1980).
18 G. M. Bell, A. Doig, M. L. Watson, A. L. Muir and R. J. Winney, *Postgrad. Med. J., 58,* 419 (1982).
19 A. Mimran and B. Jover, *Br. J. Clin. Pharmacol., 14,* 81S (1982).
20 E. D. Frohlich, R. A. Cooper and E. J. Lewis, *Arch. Intern. Med., 144,* 1441 (1984).
21 R. A. Cooper, *Arch. Intern. Med., 143,* 659 (1983).
22 B. C. Campbell, A. N. Shepherd, H. L. Elliott, K. McLean and J. L. Reid, *Br. J. Clin. Pharmacol., 13,* 755 (1982).
23 J. M. T. Willoughby, *Adverse Drug React. Bull., 100,* 368 (1983).
24 L. Knudsen and K. Weismann, *Acta Med. Scand., 204,* 75 (1978).
25 P. B. Halverson, F. Kozin, G. C. Bernhard and A. L. Goldman, *J. Am. Med. Assoc., 240,* 1870 (1978).
26 B. Amor, C. Mery and A. De Gery, *Rev. Rhum, 47,* 157 (1980).
27 G. Pasero, P. Pellegrini, M. L. Ciompi, V. Colamussi, P. Barbieri and M.-R. Mazzoni, *Rev. Rhum., 47,* 163 (1980).
28 R. I. Henkin, H. R. Keiser, I. A. Jaffe, I. Sternlieb and I. H. Scheinberg, *Lancet, II,* 1268 (1967).
29 R. I. Henkin and D. F. Bradley, *Life Sci., 9,* 701 (1970).
30 R. I. Henkin, P. J. Schechter, R. Hoye and C. F. T. Mattern, *J. Am. Med. Assoc., 217,* 434 (1971).
31 R. I. Henkin, C. W. Mueller and R. O. Wolf, *J. Lab. Clin. Med., 86,* 175 (1975).
32 R. I. Henkin, P. J. Schechter, W. T. Friedewald, D. L. Demets and M. S. Roff, *Am. J. Med. Sci., 272,* 285 (1976).

33 R. I. Henkin, in M. Kirchgessner (ed.) 'Trace Element Metabolism in Man and Animal', 3rd Symp., Arbeitskreis für Tierernahrungsforschung, (ATW), Freising, Weihenstephan, 1977.
34 F. A. Catalanotto, *Am. J. Clin. Nutr., 31,* 1098 (1978).
35 P. L. McWilliams, R. P. Agarwal and R. I. Henkin, *Biol. Trace Elements Res., 5,* 1 (1983).
36 R. I. Henkin and D. F. Bradley, *Proc. Natl. Acad. Sci. U.S.A., 62,* 30 (1969).
37 A. Cordano, in K. M. Hambidge and B. L. Nichols, Jr. (eds.), 'Zinc and Copper in Clinical Medicine', Spectrum, New York, 1978.
38 K. M. Hambidge, *Phil. Trans. R. Soc. London, Ser. B:, 294,* 129 (1981).
39 R. I. Henkin and R. L. Aamodt, in G. E. Inglett (ed.), 'Nutritional Availability of Zinc', ACS Symposium Series, 1983.
40 K. Gibbs and J. M. Walshe, *Q. J. Med., 158,* 275 (1971).
41 B. K. Park, P. S. Grabowski, J. H. K. Yeung and A. M. Breckenridge, *Biochem. Pharmacol., 31,* 1755 (1982).
42 S. J. Hoorntje, J. J. Weening, T. H. The, C. G. M. Kallenberg, J. M. A. Donker and P. J. Hoedemaeker, *Lancet, I,* 1212 (1980).
43 J. K. Wilkin, J. J. Hammond and W. M. Kirkendall, *Arch. Dermatol., 116,* 902 (1980).
44 J. R. Luderer, D. P. Lookingbill, D. W. Schneck, L. M. Demers, C. Cohen and A. H Hayes, Jr., *J. Clin. Pharmacol., 22,* 151 (1982).
45 P. A. Kendall and D. Hutchins, *Immunology, 35,* 189 (1978).
46 P. E. Lipsky and M. Ziff, *J. Clin. Invest., 65,* 1069 (1980).
47 P. E. Lipsky, *Agents Actions Suppl., 8,* 85 (1980).
48 A. I. Vogel, in 'Textbook for Quantitative Inorganic Analysis', Longman, London, 1961, p. 434.
49 P. M. May, D. R. Williams, P. W. Linder and R. G. Torrington, *Talanta, 29,* 249 (1982).
50 G. Berthon, P. M. May and D. R. Williams, *J. Chem. Soc., Dalton Trans.,* 1434 (1978).
51 A. Sabatini, A. Vacca and P. Gans, *Talanta, 21,* 53 (1974).
52 D. R. Williams, *J. Chem. Educ., 48,* 480 (1971).
53 D. R. Williams, *J. Chem. Soc., Dalton Trans.,* 1064 (1973).
54 K. M. Murray and P. M. May, 'ESTA Users Manual', UWIST, Cardiff, 1984.
55 P. M. May, P. W. Linder and D. R. Williams, *J. Chem. Soc., Dalton Trans.,* 588 (1977).
56 D. D. Perrin and I. G. Sayce, *J. Chem. Soc. A:,* 82 (1967).
57 A. Avdeef and D. L. Kearvey, *J. Am. Chem. Soc., 104,* 7212 (1982).
58 K. K. Wong, S. J. Lan and B. H. Migdalof, *Biochem. Pharmacol., 30,* 2643 (1981).
59 K. J. Kripalani, D. N. McKinstry, S. M. Singhvi, D. A. Willard, R. A. Vukovich and B. H. Migdalof, *Clin. Pharmacol. Ther., 27,* 636 (1980).
60 R. J. Cody, G. L. Schaer, A. B. Covit, K. Pondolfino and G. Williams, *Clin. Pharmacol. Ther., 32,* 721 (1982).
61 R. Jarrott, A. Anderson, R. Hooper and W. J. Louis, *J. Pharm. Sci., 70,* 665 (1981).
62 A. J. Rommel, A. M. Pierides and A. F. Heald, *Clin. Pharmacol. Ther., 27,* 282 (1980).
63 B. Jarrott, O. H. Drummer, R. Hooper, A. I. E. Anderson and W. J. Louis, *Am. J. Cardiol., 49,* 1547 (1982).
64 K. L. Duchin, S. M. Singhvi, D. A. Willard, B. H. Migdalof and D. N. McKinstry, *Clin. Pharmacol. Ther., 31,* 452 (1982).
65 B. H. Migdalof, K. K. Wong, S. J. Lan, K. J. Kripalani and S. M. Singhvi, *Fed. Proc., 39,* 757 (1980).
66 S. J. Lan, S. H. Weinstein and B. H. Migdalof, *Drug Metab. Disp., 10,* 306 (1982).
67 A. F. Heald and C. E. Ita, *Pharmacologist, 19,* 129 (1977).
68 R. I. Henkin, P. J. Schechter, M. S. Roff, D. A. Bronzert and W. T. Friedewald, in W. Pories and W. H. Strain, (eds.), 'Clinical Applications of Zinc Metabolism', Thomas Springfield, Ill., 1974, p. 204.
69 F. M. Andrews, D. N. Golding, A. M. Freeman, J. R. Golding, A. T. Day, A. G. S. Hill, A. V. Camp, E. Lewis-Faning and W. H. Lyle, *Lancet, I,* 275 (1973).
70 I. A. Jaffe, *Postgrad. Med. J., Suppl.,* 15 (1968).
71 I. A. Jaffe, *Arthritis Rheum., 13,* 436 (1970).
72 H. R. Keiser, R. I. Henkin and M. Kare, *Proc. Soc. Exp. Biol. Med., 129,* 516 (1968).
73 J. J. McNeil, A. Anderson, N. Christophidis, B. Jarrott and W. J. Louis, *Br. Med. J., 2,* 1555 (1979).
74 G. A. MacGregor, N. D. Markandu, J. E. Roulston and J. C. Jones, *Br. Med. J., 2,* 1106 (1979).
75 A. J. Smit, S. J. Hoorntje and A. J. M. Donker, *Nephron, 34,* 196 (1983).
76 Y. Sugiura and H. Tanaka, *Chem. Pharm. Bull. (Tokyo), 18,* 368 (1970).
77 W. K. Musker and C. H. Neagley, *Inorg. Chem., 14,* 1728 (1975).
78 R. G. Fassett, R. G. Walker, J. A. Whitworth and P. Kincaid-Smith, *Br. Med. J., 286,* 648 (1983).
79 A. Gergely and I. Sóvágó, *Bioinorg. Chem., 9,* 47 (1978).
80 S. H. Laurie, T. Lund and J. B. Raynor, *J. Chem. Soc., Dalton Trans.,* 1389 (1975).
81 I. Sóvágó and A. Gergely, *Agents Actions Suppl., 8,* 291 (1980).