Syntheses of Macrocyclic Enzyme Models. Part 5.† Preparation and Metal-co-ordination Behaviour of [20]Paracyclophane bearing 1,4-Dihydronicotinamide and Pyridine-2-carboxylic Acid Moieties as an Alcohol Dehydrogenase Model

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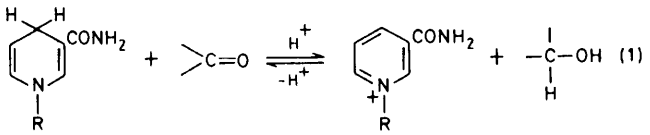
A [20]paracyclophane (PCP) bearing 1,4-dihydronicotinamide (HNA) on the benzene ring and pyridine-2-carboxylic acid (Py) as a metal-binding ligand on C-10 of the macrocycle *via* an amide linkage, HNA–PCP–Py, has been prepared as a model for the NAD-dependent alcohol dehydrogenase. Its co-ordination behaviour with zinc(II) ion in dichloromethane–methanol and dichloromethane–propan-2-ol (100 : 1 v/v) has been investigated and compared with those of PCP–HNA and PCP–Py. PCP–Py forms zinc complexes having 2 : 1 and 1 : 1 (PCP–Py to Zn^{II}) stoicheiometry, the 2 : 1 complex predominating over the 1 : 1 complex in stability. PCP–HNA also forms a relatively stable 1 : 1 complex with zinc ion (PCP–HNA–Zn^{II}), in which the dihydronicotinamide moiety provides a metal-binding site, with a stability constant an order of magnitude larger than the overall stability constant for PCP–Py–Zn^{II}. The major zinc-bound species of HNA–PCP–Py is a 1 : 1 complex (PCP$<^{Py}_{HNA}>Zn^{II}$), in which both Py and HNA moieties are simultaneously co-ordinated to the same zinc ion. Detailed analysis of the co-ordination equilibria indicates that the 2 : 1 complex (HNA–PCP–Py–Zn^{II}–Py–PCP–HNA), in which HNA is free from metal co-ordination, provides only a minor contribution to the overall zinc co-ordination scheme.

THE NAD-dependent alcohol dehydrogenase contains zinc ion as an essential cofactor. It is generally recognized that direct co-ordination of the enzyme-bound zinc ion to the carbonyl and hydroxy groups of substrates promotes coenzyme–substrate hydrogen transfer [equation (1)] by polarizing the carbonyl group and by facilitating deprotonation of the hydroxy-group, respectively.[1-4] Recent model studies, which take into account



(1)

zinc-ion participation in enzyme functions, have been directed towards the development of systems involving a simple 1,4-dihydronicotinamide, a chelating carbonyl substrate, and zinc ion:[5-15] zinc complexes of chelating carbonyl substrates are far more susceptible (relative to the metal-free species) to reduction by dihydronicotinamides. It should be emphasized, however, that a good alcohol dehydrogenase model should exhibit a substrate–zinc interaction arising from an enforced proximity effect even if the substrate has no or little intrinsic capability of co-ordinating with zinc ion; this will occur if the dihydronicotinamide and the zinc ion are in an appropriate and fixed orientation owing to some rigid framework. Therefore, we have taken an alternative approach along this line, and the molecular design of a macrocyclic enzyme model, which contains both nicotinamide and an intramolecular zinc-binding site, has been now made.

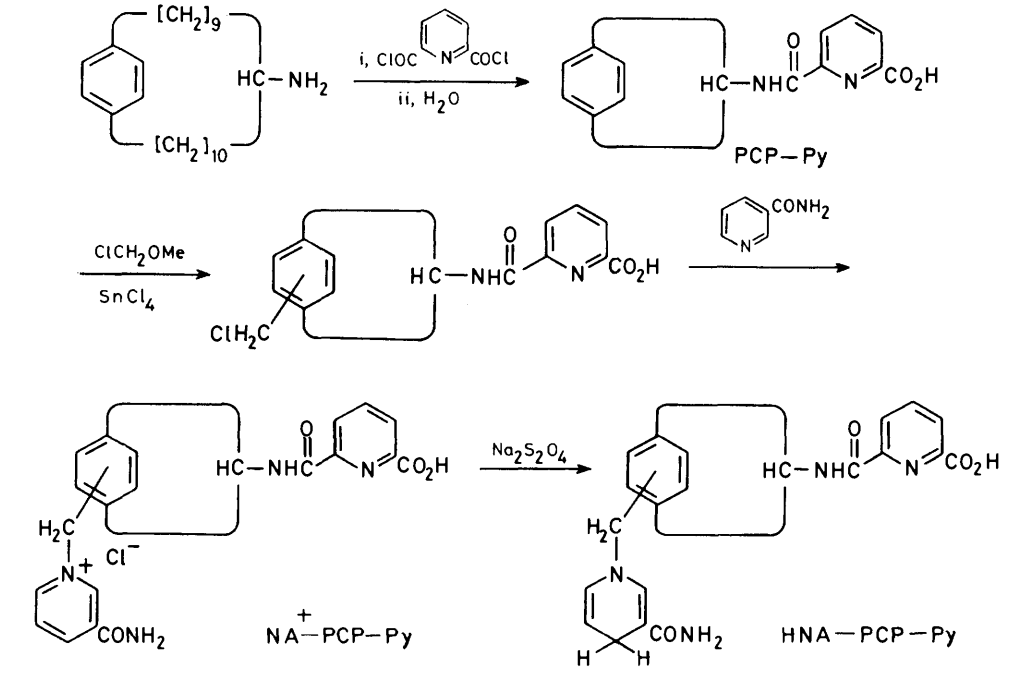
Previous papers in this series have characterized [20]- and [10.10]-paracyclophanes as efficient enzyme models with regard to their hydrophobic and stereo-regulative abilities.[16-25] Consequently, we selected specifically [20]paracyclophane as a basic macrocyclic skeleton and incorporated covalently dihydronicotinamide and pyridine-2-carboxylic acid moieties into it. The purpose of the present study is to clarify the zinc-co-ordination behaviour of the [20]paracyclophane-based alcohol dehydrogenase model in relation to those of related compounds having only one of the constituents, the dihydronicotinamide or pyridine-2-carboxylic acid moiety.

RESULTS AND DISCUSSION

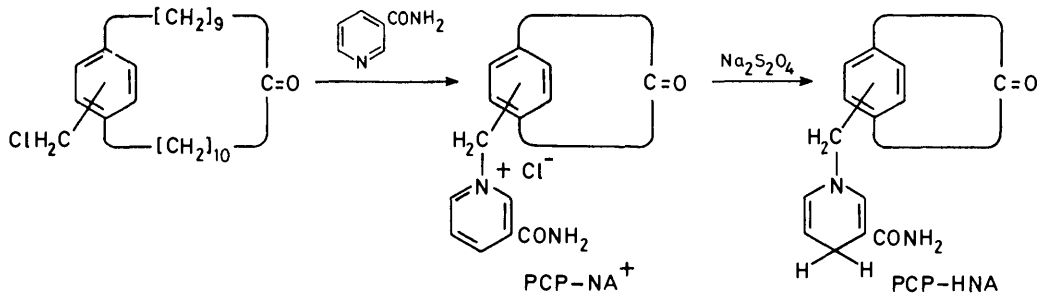
Preparation.—A [20]paracyclophane bearing a 1,4-dihydronicotinamide moiety (abbreviated as HNA) on the benzene ring and pyridine-2-carboxylic acid (abbreviated as Py) connected to C-10 of the macrocyclic skeleton through an amide linkage was prepared (Scheme 1; product abbreviated as HNA–PCP–Py). The condensation of 10-amino[20]paracyclophane with pyridine-2,6-dicarbonyl dichloride followed by hydrolysis afforded the monoamide (PCP–Py). The amide (PCP–Py) was converted into the chloromethyl derivative which, on treatment with nicotinamide, gave the nicotinamide salt (NA^+–PCP–Py). Reduction of NA^+–PCP–Py to the corresponding 1,4-dihydronicotinamide (HNA–PCP–Py) was carried out most efficiently with sodium dithionite in an aqueous phosphate buffer (pH 7).[26] As a reference compound, an *N*-cyclophanylmethyl-1,4-dihydronicotinamide (PCP–HNA) lacking a pyridine-2-carboxylic acid moiety was also prepared by quaternization of nicotinamide with 22(23)-chloromethyl[20]paracyclophan-10-one followed by reduction with sodium dithionite (Scheme 2).

Metal-co-ordination Behaviour.—The metal-ion co-ordination at the pyridine-2-carboxylic acid moiety of PCP–Py and HNA–PCP–Py was confirmed by the spec-

† Part 4, Y. Murakami, A. Nakano, K. Akiyoshi, and K. Fukuya, preceding paper.



SCHEME 1



SCHEME 2

tral changes observed for both the pyridine chromophore and the metal ion. In the n.m.r. spectrum of PCP–Py (2.0×10^{-2} mol dm^{-3} in [$^2H_4$]methanol), only the pyridine proton signals exhibited downfield shifts, by *ca.* 0.3 p.p.m., upon addition of $ZnCl_2$ (1.0×10^{-1} mol dm^{-3}, 10-fold excess), other resonances being unaffected.[27] The e.s.r. spectrum for a frozen solution of $CuCl_2$ (1.0×10^{-2} mol dm^{-3} in ethanol) at 77 K gives spin Hamiltonian parameters $g_{\|} = 2.349$ and $A_{\|}^{Cu} = 120 \times 10^{-4}$ cm^{-1}. In the presence of an equimolar amount of PCP–Py, another set of hyperfine splittings was observed in the $g_{\|}$ component indicative of a new Cu^{II} species ($g_{\|} = 2.308$ and $A_{\|}^{Cu} = 91.5 \times 10^{-4}$ cm^{-1}), which predominated when the molar ratio of PCP–Py : Cu^{II} was raised to 10. The π–π^* transition bands appearing over a 275 nm range due to the pyridine chromophore of PCP–Py (5.0×10^{-5} or 1.0×10^{-4} mol dm^{-3}) in dichloromethane–methanol (100 : 1 v/v) or dichloromethane–propan-2-ol (100 : 1 v/v) increased their intensities upon addition of $ZnCl_2$ (Figure 1; measured in dichloromethane–propan-2-ol). The simplest co-ordination equilibrium would involve a 1 : 1 (PCP–Py : Zn^{II}) complex formation [equation (2)]. If this is the case, the ratio [PCP–Py–Zn^{II}]/[PCP–Py][Zn^{II}], readily evaluated from

$$\text{PCP–Py} \underset{K_{11}}{\overset{Zn^{II}}{\rightleftharpoons}} \text{PCP–Py–}Zn^{II} \qquad (2)$$

the spectral change and the known amount of Zn^{II}, must be constant; the ratio, however, decreases with increase in [$ZnCl_2$] as can be seen in Table 1. This suggests that the zinc-binding scheme is not a simple one, but involves the formation of both 2 : 1 and 1 : 1 (PCP–Py/Zn^{II}) species [equation (3)]. The observation of an

$$2(\text{PCP–Py}) \underset{K_1}{\overset{Zn^{II}}{\rightleftharpoons}} \text{PCP–Py–}Zn^{II}\text{–Py–PCP} \underset{K_2}{\overset{Zn^{II}}{\rightleftharpoons}} 2(\text{PCP–Py–}Zn^{II}) \qquad (3)$$

isosbestic point (Figure 1) coupled with more direct observations made for metal complexes of a picolinic acid derivative [28] may suggest that the present 2 : 1 and 1 : 1 species have similar molar absorption coefficients per PCP–Py unit. For practical purposes, their identity was assumed here and the respective stability constants (K_1 and K_2) were evaluated by least-squares analysis

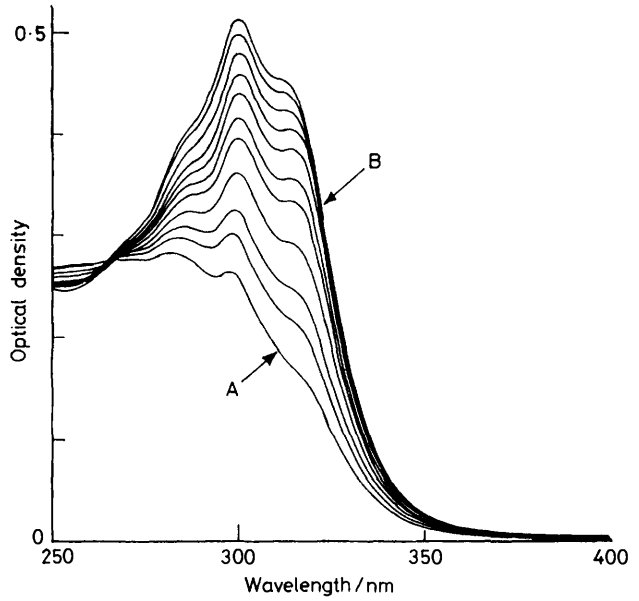


FIGURE 1 Electronic spectra of PCP–Py (5.0×10^{-5} mol dm^{-3}) in dichloromethane–propan-2-ol (100 : 1 v/v) at 25.0 °C in the presence of varying amounts of $ZnCl_2$. $[ZnCl_2]$: 0, 2.0×10^{-5}, 4.0×10^{-5}, 8.0×10^{-5}, 1.2×10^{-4}, 2.0×10^{-4}, 4.0×10^{-4} 8.0×10^{-4}, 1.2×10^{-3}, 2.0×10^{-3}, and 4.0×10^{-3} mol dm^{-3}; read from A to B

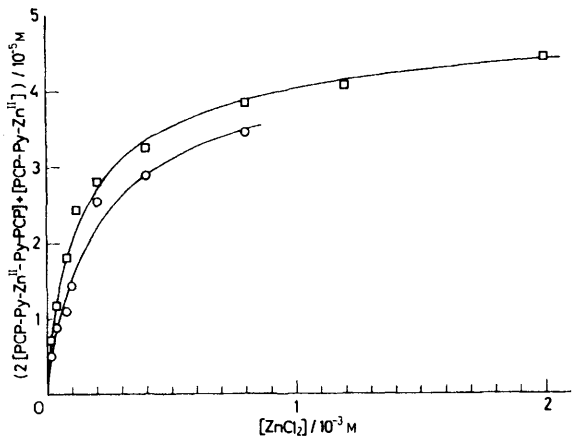


FIGURE 2 Correlations between total concentrations of $ZnCl_2$ and (PCP–Py)–Zn^{II} complexes; experimentally observed in dichloromethane–methanol (100 : 1 v/v) (○) and in dichloromethane–propan-2-ol (100 : 1 v/v) (□) at 25.0 °C. Solid lines refer to calculated data on the basis of equation (3) and evaluated stability constants; total concentration of PCP–Py, 5.0×10^{-5} mol dm^{-3}

greater than that of the 1 : 1 complex over a wide range of zinc concentration. This becomes clearer when the equilibrium constant for co-ordination of free PCP–Py with free zinc ($ZnCl_2$) [equation (2)] is compared with that for free PCP–Py to co-ordinate with PCP–Py-bound zinc [equation (4)]: $K' = (K_1/K_2)^{\frac{1}{2}}$ 3.4×10^4 and 4.1×10^4 in dichloromethane–methanol and dichloromethane–propan-2-ol, respectively. Thus, the 1 : 1 complex has a greater tendency to bind free PCP–Py than free $ZnCl_2$ by a factor of *ca.* 20.

(see Experimental section): in dichloromethane–methanol (100 : 1 v/v) $K_1 = 5.0 \times 10^7$ mol^{-2} dm^6, $K_2 = 4.3 \times 10^{-2}$, and the overall stability constant $K_{11} = (K_1K_2)^{\frac{1}{2}} = 1.5 \times 10^3$ mol^{-1} dm^3; in dichloromethane–propan-2-ol (100 : 1 v/v) $K_1 = 9.3 \times 10^7$ mol^{-2} dm^6, $K_2 = 5.6 \times 10^{-2}$, and $K_{11} = 2.3 \times 10^3$ mol^{-1} dm^3. The observed total concentration of zinc–cyclophane complexes in terms of 2[PCP–Py–Zn^{II}–Py–PCP] + [PCP–Py–Zn^{II}] can be reproduced quite satisfactorily by calculations (see Experimental section) based on equation (3), using K_1 and K_2 values determined as above, for the whole concentration range of $ZnCl_2$ employed in the present study (Figure 2 for the solvent systems of dichloromethane–methanol and dichloromethane–propan-2-ol).

In Figure 3 are shown the calculated molar distributions of the metal-free, 2 : 1 (in PCP–Py unit), and 1 : 1 species of PCP–Py in dichloromethane–methanol (100 : 1 v/v) as a function of $ZnCl_2$ concentration. Somewhat surprisingly, the proportion of the 2 : 1 complex is

$$\text{PCP–Py} + \text{PCP–Py–Zn}^{II} \underset{}{\overset{K'}{\rightleftharpoons}} \text{PCP–Py–Zn}^{II}\text{–Py–PCP} \quad (4)$$

Spectroscopic evidence indicates that 1,4-dihydronicotinamide forms a zinc complex in aprotic solvents.[7,29] Upon addition of $ZnCl_2$ in dichloromethane–methanol (100 : 1 v/v), the absorption maximum of PCP–HNA (1.0×10^{-4} mol dm^{-3}) at 350 nm undergoes a red shift with concomitant increase in intensity (Figure 4). The spectral change is consistent with formation of the 1 : 1 complex [equation (5)] since the ratio [PCP–HNA–Zn^{II}]/[PCP–HNA][Zn^{II}] is practically constant regard-

TABLE 1

Analysis of spectral data for the PCP–Py–$ZnCl_2$ system in dichloromethane–propan-2-ol (100 : 1 v/v) at 25 °C [a]

$10^5[ZnCl_2]_0$/ mol dm^{-3}	10^5[PCP–Py–Zn^{II}]/ mol dm^{-3}	10^5[PCP–Py]/ mol dm^{-3}	$10^5[Zn^{II}]$/ mol dm^{-3}	$\frac{10^{-3}[\text{PCP–Py–Zn}^{II}]}{[\text{PCP–Py}][Zn^{II}]}$/mol^{-1} dm^3
2	0.72	4.28	1.28	13
4	1.19	3.81	2.81	11
8	1.80	3.20	6.20	9.1
12	2.44	2.56	9.56	10
20	2.81	2.19	17.19	7.5
40	3.26	1.74	36.74	5.1
80	3.84	1.16	76.16	4.3
120	4.05	0.95	115.9	3.7
200	4.42	0.58	195.6	3.9

[a] $[\text{PCP–Py}]_0 = 5.0 \times 10^{-5}$ mol dm^{-3}; [PCP–Py] = $[\text{PCP–Py}]_0$ − [PCP–Py–Zn^{II}]; $[Zn^{II}] = [ZnCl_2]_0$ − [PCP–Py–Zn^{II}].

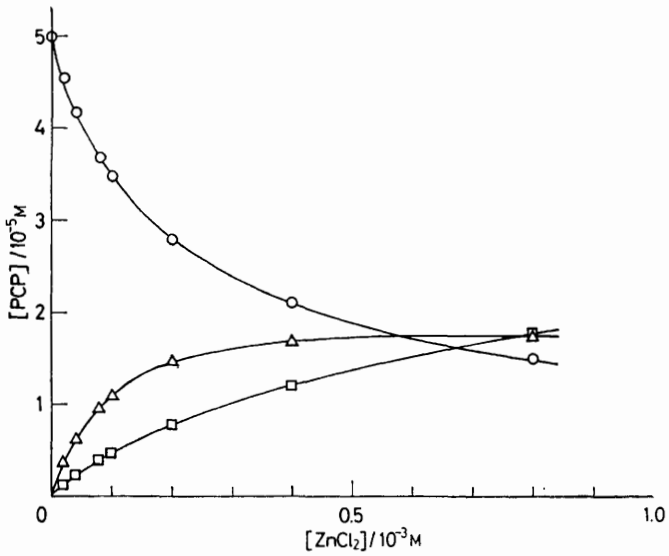


FIGURE 3 Distributions of PCP–Py (○), PCP–Py–Zn^{II}–Py–PCP (△) (in PCP–Py unit), and PCP–Py–Zn^{II} (□) as functions of total concentration of $ZnCl_2$ in dichloromethane–methanol (100 : 1 v/v); total concentration of PCP–Py, 5.0×10^{-6} mol dm^{-3}

less of total zinc concentration, in contrast to the case for PCP–Py; the stability constant is 1.2×10^4 mol^{-1} dm^3, one order of magnitude larger than the overall stability constant for zinc-ion co-ordination with the pyridine-2-carboxylic acid moiety of PCP–Py in the same medium.

$$PCP\text{–}HNA + Zn^{II} \rightleftharpoons PCP\text{–}HNA\text{–}Zn^{II} \quad (5)$$

The co-ordination behaviour of the bifunctional cyclophane (HNA–PCP–Py) was next examined. The spectral change in dichloromethane–methanol (100 : 1 v/v) is shown in Figure 5. The spectral change at 350 nm

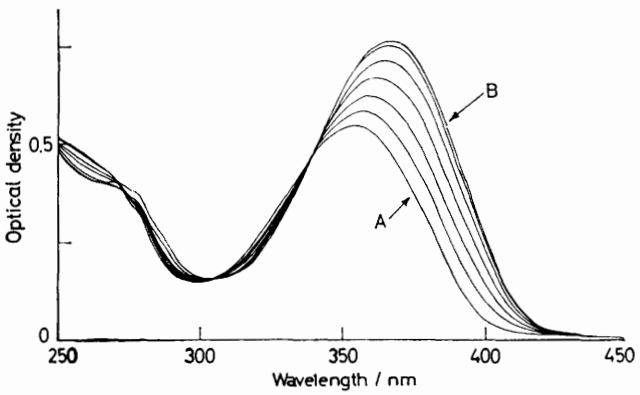


FIGURE 4 Electronic spectra of PCP–HNA (1.0×10^{-4} mol dm^{-3}) in dichloromethane–methanol (100 : 1 v/v) at 25.0 °C in the presence of varying amounts of $ZnCl_2$. [$ZnCl_2$]: 0. 5.0×10^{-5}, 1.0×10^{-4}, 2.0×10^{-4}, 4.0×10^{-4}, 1.0×10^{-3}, and 2.0×10^{-3} mol dm^{-3}; read from A to B

as a measure of zinc co-ordination with the dihydronicotinamide moiety and that at 275 nm as a measure of zinc co-ordination with the pyridine-2-carboxylic acid moiety are nearly comparable to each other and are consistent with formation of a complex with 1 : 1 (HNA–PCP–Py to Zn^{II}) stoicheiometry; *i.e.* the ratio [(HNA–PCP–Py) Zn^{II}]/[HNA-PCP–Py][Zn^{II}] is nearly constant over a wide range of zinc concentration, the apparent stability constant being 1.1×10^4 mol^{-1} dm^3. This value should be corrected for the reasons described below. As regards the co-ordination tendency of the dihydronicotinamide site towards zinc, there is no big difference

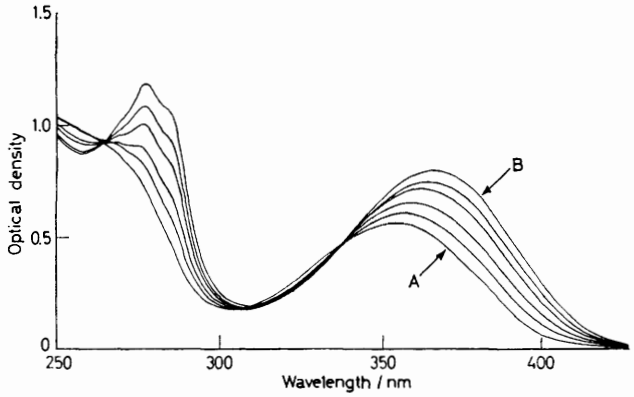
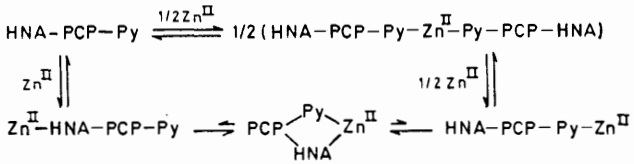


FIGURE 5 Electronic spectra of HNA–PCP–Py (1.0×10^{-4} mol dm^{-3}) in dichloromethane–methanol (100 : 1 v/v) at 25.0 °C in the presence of varying amounts of $ZnCl_2$. [$ZnCl_2$]: 0, 5.0×10^{-5}, 1.0×10^{-4}, 2.0×10^{-4}, 4.0×10^{-4}, and 1.0×10^{-3} mol dm^{-3}; read from A to B

between HNA–PCP–Py and PCP–HNA. However, for the co-ordination of zinc ion to the pyridine-2-carboxylic acid moiety, a definite change in stoicheiometry is observed on changing the ligand from PCP–Py to HNA–PCP–Py; the 2 : 1 complex prevails over the 1 : 1 complex in stability for the former, while the latter yields primarily the 1 : 1 complex. The tendency of HNA–PCP–Py to co-ordinate to zinc is enhanced to such an extent that the pyridine-2-carboxylic acid and dihydronicotinamide moieties in HNA–PCP–Py have nearly the same zinc-binding ability. These results clearly indicate that the zinc ion forms predominantly a 1 : 1 complex (I) with HNA–PCP–Py which acts as a macrocyclic chelating ligand (Scheme 3). This seems to



(I)

SCHEME 3

be the first spectroscopic evidence for the formation of a metal chelate structure with such a macrocycle as [20]paracyclophane.* Turning to the minor zinc-binding species (Scheme 3), the most important one is the 2 : 1 complex (HNA–PCP–Py–Zn^{II}–Py–PCP–HNA) in which the dihydronicotinamide moiety is free from

* We have also suggested a similar chelating structure from kinetic evidence for the complex formed between copper(II) ion and the di-imidazole derivative of [10.10]paracyclophane.[25]

metal-co-ordination.* Its accurate concentrations could not be determined directly but were evaluated as follows. (*i*) Analysis of the 350 nm spectral change allows direct determination of the concentration of the dihydronicotinamide–Zn^{II} species [mainly structure (1) and Zn^{II}–HNA–PCP–Py as a minor component if any]. (*ii*) The remaining Zn^{II} and HNA–PCP–Py are in equilibrium with the 2 : 1 complex [equation (3)] in which the dihydronicotinamide moiety is metal-free; the stability constant is assumed to be comparable to that for the PCP–Py complex (5.0×10^7 mol^{-2} dm^6). In Figure 6

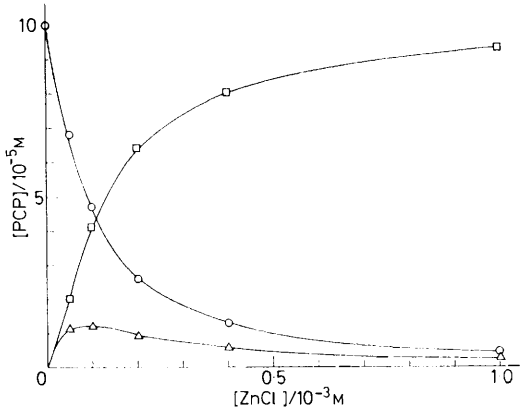


FIGURE 6 Distributions of HNA–PCP–Py (○), PCP$\langle^{Py}_{HNA}\rangle Zn^{II}$ (□), and HNA–PCP–Py–Zn^{II}–Py–PCP–HNA (△) (in HNA–PCP–Py unit) as functions of total concentration of $ZnCl_2$ in dichloromethane–methanol (100 : 1 v/v); total concentration of HNA–PCP–Py, 1.0×10^{-4} mol dm^{-3}

are shown the molar distributions of the metal-free, 1 : 1 [mainly structure (1)], and 2 : 1 (in the ligand unit) species evaluated along these lines as a function of $ZnCl_2$ concentration. The fraction of the 2 : 1 species reaches a maximum and falls as the $ZnCl_2$ concentration increases. The stability constant of the 1 : 1 complex needs to be corrected for the amount of zinc ion consumed in the formation of the 2 : 1 complex, the corrected value being 1.7×10^4 mol^{-1} dm^3. The stability constants are summarized in Table 2.

In conclusion, both dihydronicotinamide and pyridine-2-carboxylic acid moieties in HNA–PCP–Py have comparable zinc-binding abilities and form a macrocyclic chelate in a 1 : 1 molar ratio (HNA–PCP–Py : zinc). While HNA–PCP–Py and PCP–HNA form the 1 : 1 zinc complex with similar co-ordination tendencies, the 2 : 1 (ligand : zinc) complex prevails in stability over the 1 : 1 complex when PCP–Py is used as a ligand. On the other hand, the 2 : 1 complex of HNA–PCP–Py to zinc, in which the dihydronicotinamide moiety is free from metal-co-ordination, contributes only a minor fraction to the whole co-ordination equilibria.

* Preliminary work on the reaction between the present cyclophane-1,4-dihydronicotinamide (PCP–HNA or HNA–PCP–Py, 1.0×10^{-4} mol dm^{-3}) and hexachloroacetone (1.0×10^{-2} mol dm^{-3}) in dichloromethane–methanol (100 : 1 v/v) has been carried out both in the presence and absence of $ZnCl_2$. The rate of reaction with PCP–HNA steadily decreased as the concentration of Zn^{II} increased until it levelled off beyond $[Zn^{II}] > 1 \times 10^{-3}$ mol dm^{-3}. On the other hand, the reaction rate in the presence of HNA–PCP–Py reached a maximum at lower Zn^{II} concentration (*ca.* 1.0×10^{-4} mol dm^{-3}) and then fell as $[Zn^{II}]$ increased. Kinetic analysis indicates as follows: the 2 : 1 complex, HNA–PCP–Py–Zn^{II}–Py–PCP–HNA, which formed as a minor fraction, exercises a much enhanced activity (seven times as reactive as metal-free HNA–PCP–Py), while the 1 : 1 complex as a major fraction has somewhat reduced reactivity relative to metal-free HNA–PCP–Py.

TABLE 2

Stability constants for zinc complexes of PCP–Py, PCP–HNA, and HNA–PCP–Py at 25.0 °C

Equilibrium	Medium [a]	K
2 (PCP–Py) $\overset{Zn^{II}}{\rightleftharpoons}$ PCP–Py–Zn^{II}–Py–PCP	A	5.0×10^7 mol^{-2} dm^6
	B	9.3×10^7 mol^{-2} dm^6
PCP–Py–Zn^{II}–Py–PCP $\overset{Zn^{II}}{\rightleftharpoons}$ 2 (PCP–Py–Zn^{II})	A	0.043
	B	0.056
PCP–Py $\overset{Zn^{II}}{\rightleftharpoons}$ PCP–Py–Zn^{II}	A	1.5×10^3 mol^{-1} dm^3
	B	2.3×10^3 mol^{-1} dm^3
PCP–Py + PCP–Py–Zn^{II} $\rightleftharpoons$ PCP–Py–Zn^{II}–Py–PCP	A	3.4×10^4 mol^{-1} dm^3
	B	4.1×10^4 mol^{-1} dm^3
PCP–HNA $\overset{Zn^{II}}{\rightleftharpoons}$ PCP–HNA–Zn^{II}	A	1.2×10^4 mol^{-1} dm^3
HNA–PCP–Py $\overset{Zn^{II}}{\rightleftharpoons}$ PCP$\langle^{Py}_{HNA}\rangle Zn^{II}$	A	1.7×10^4 mol^{-1} dm^3
2 (HNA–PCP–Py) $\overset{Zn^{II}}{\rightleftharpoons}$ HNA–PCP–Py–Zn^{II}–Py–PCP–HNA	A	5.0×10^7 mol^{-1} dm^3 [b]

[a] A, dichloromethane–methanol (100 : 1 v/v); B, dichloromethane–propan-2-ol (100 : 1 v/v). [b] Assumed.

EXPERIMENTAL

I.r. spectra were recorded on a JASCO IR-E spectrophotometer. ^{1}H N.m.r. spectra were taken on a Varian A-60, a Bruker WH-90 FT, or a JEOL FX-100 spectrometer with tetramethylsilane as an internal reference. Fluorescence and electronic absorption spectra were obtained with a Shimadzu spectrofluorophotometer RF-500 and a Union Giken SM-401 high sensitivity spectrophotometer, respectively. E.s.r. spectra were measured with a JEOL JES-ME-3 X-band spectrometer by using the manganese(II) ion, diffused thermally into magnesium oxide, as reference. High performance liquid chromatography for preparative purposes were carried out on a Hitachi 635 liquid chromatograph with Hitachi gel 3019. Gel-filtration chromatography was performed on a column packed with Sephadex LH-20. Methanol was used as eluant unless otherwise indicated, and components eluted were detected by u.v. absorption at either 254 or 265 nm for both chromatographic techniques. Dichloromethane for metal-co-ordination studies was fractionally distilled and stored over molecular sieves. Zinc chloride of the highest quality was fused just before use.

10-(2-*Carboxypyridine*-6-*carboxamido*)[20]*paracyclophane* (*PCP–Py*).—Into a refluxing solution of pyridine-2,6-dicarbonyl dichloride (7.8 g, 38 mmol) and pyridine (3.0 g, 38 mmol) in benzene (30 ml) was added dropwise a benzene solution (100 ml) of 10-amino[20]paracyclophane [30] (1.88 g, 5.1 mmol) over 6 h. The mixture was further stirred under reflux for 1 h and cooled down to room temperature.

Water (100 ml) was added and the mixture was stirred for 90 min. The organic layer was separated and the aqueous layer was extracted with ether (100 ml × 4). The organic layer and the extract were combined, washed with water, and dried (Na_2SO_4). The solvent was evaporated off and the residue was chromatographed on a column of silica gel (Wako gel C-100) with dichloromethane (1 600 ml) as eluant. After evaporation of the solvent, the residue was recrystallized from ethyl acetate–light petroleum to give *PCP–Py* (370 mg, 14%), m.p. 112.7—113.8 °C; $\nu_{max.}$ (KBr) 3 250 (NH), 1 753 (carboxy C=O), 1 650 (amide C=O), and 1 530 cm^{-1} (NH); δ (CD_3OD) 8.04—8.48 (3 H, m, Hs on pyridine ring), 7.12 (4 H, s, Hs on benzene ring), 4.10 (1 H, m, *H*CNH), 2.62 (4 H, t, benzyl), and 0.8—2.0 (34 H, m, methylene Hs) (Found: C, 75.7; H, 9.3; N, 5.25. $C_{33}H_{48}N_2O_3$ requires C, 76.1; H, 9.3; N, 5.4%).

N-{10(11)-(2-*Carboxypyridine*-6-*carboxamido*)[20]*paracyclophan*-22-*ylmethyl*}*nicotinamide Chloride* (*NA*$^+$–*PCP–Py*).—Into a solution of PCP–Py (200 mg, 4.3 mmol) and chloromethyl methyl ether (350 mg, 4.3 mmol) in dichloromethane (20 ml) on an ice-bath was added a dichloromethane solution (10 ml) of anhydrous tin(IV) chloride (350 mg, 1.34 mmol) over 90 min. The mixture was stirred for another 90 min, poured onto ice–water (100 g) containing concentrated hydrochloric acid (10 ml), and extracted with dichloromethane (50 ml × 3). The extract was washed with water, dried (Na_2SO_4), and evaporated to give the chloromethylated derivative as an oil (177 mg, 81%); $\nu_{max.}$ (neat) 1 260 (CH of CH_2Cl) and 740 cm^{-1} (CCl); δ ($CDCl_3$) 8.0—8.7 (4 H, m, Hs on pyridine ring and CO_2H), 7.13 (3 H, m, Hs on benzene ring), 4.67 (2 H, s, CH_2Cl), 4.1br (1 H, s, *H*CNH), 2.61 (4 H, t, benzyl), and 0.8—2.0 (34 H, m, methylene Hs). A solution of the chloromethylated derivative (177 mg, 0.31 mmol) and nicotinamide (100 mg, 0.82 mmol) in ethanol (20 ml) was refluxed for 4 h. The solvent was evaporated off and the oily residue was subjected to repeated chromatographic separation (gel filtration, h.p.l.c. and further gel filtration) to afford NA$^+$–PCP–Py as a *glass* (62 mg, 29%); δ (CD_3OD) 9.55 (1 H, s, 2-H on nicotinamide ring), 9.15 (2 H, d, 4- and 6-H on nicotinamide ring), 8.0—8.5 (4 H, m, 5-H on nicotinamide ring and Hs on pyridine ring), 7.33 (3 H, m, Hs on benzene ring), 6.00 (2 H, s, CH_2N^+), 4.1br (1 H, s, *H*CNH), 2.65 (4 H, t, benzyl), and 0.8—1.9 (34 H, m, other methylene Hs) (Found: C, 69.15; H, 8.35; N, 7.6. $C_{40}H_{55}ClN_4O_4$ requires C, 69.5; H, 8.0; N, 8.1%).

N-{10(11)-*Oxo*[20]*paracyclophan*-22-*ylmethyl*}*nicotinamide Chloride* (*PCP–NA*$^+$).—A solution of 22(23)-chloromethyl-[20]paracyclophan-10-one [30] (550 mg, 1.3 mmol) and nicotinamide (350 mg, 2.9 mmol) in ethanol (20 ml) was refluxed for 6 h. Evaporation of ethanol gave an oily residue. Gel-filtration chromatography afforded a solid (313 mg), which was recrystallized from methanol-ether to give *PCP–NA*$^+$ (250 mg, 35%), m.p. 135.0—136 °C; δ (CD_3OD) 9.45 (1 H, s, 2-H on nicotinamide ring), 9.10 (2 H, d, 4- and 6-H on nicotinamide ring), 8.30 (1 H, t, 5-H on nicotinamide ring), 7.30 (3 H, d, Hs on benzene ring), 6.03 (2 H, s, CH_2N^+), 2.71 (4 H, m, benzyl), 2.40 (4 H, t, CH_2 adjacent to C=O), and 0.9—1.8 (30 H, m, other methylene Hs) (Found: C, 72.8; H, 9.1; N, 5.1. $C_{33}H_{49}ClN_2O_2$ requires C, 73.25; H, 9.15; N, 5.2%).

N-{10(11)-(2-*Carboxypyridine*-6-*carboxamido*)[20]*paracyclophan*-22-*ylmethyl*}-1,4-*dihydronicotinamide* (*HNA–PCP–Py*).—A solution of sodium dithionite (675 mg, 3.9 mmol) in 0.1 mol dm^{-3} phosphate buffer (pH 7.0, 25 ml) on an ice-bath was protected from room light and maintained under nitrogen. Upon addition in one portion of a solution of NA$^+$–PCP–Py (42 mg, 0.064 mmol) in ethanol (0.5 ml), the mixture immediately turned yellow and became turbid. After the mixture had been stirred for 30 min, dichloromethane (25 ml) was added and stirring was continued. The reaction was monitored by measuring the absorbance of an aliquot taken at appropriate time intervals. The absorbance due to the dihydronicotinamide moiety reached a maximum after 3.5 h. The organic layer was separated and the aqueous layer was extracted with dichloromethane (25 ml × 3). The organic layer and the extract were combined, washed with water containing a small amount of sodium chloride (50 ml × 3), dried over Na_2SO_4 in a refrigerator for 2.5 h, and evaporated *in vacuo*. The residue was purified by gel-filtration chromatography using dry methanol as eluant to give HNA–PCP–Py as a yellow oil (12.35 mg, 31%); $\lambda_{max.}$ [CH_2Cl_2–MeOH (100 : 1 v/v)] 354.5 nm (ε 5 600); fluorescence maximum (methanol) 446 nm (excitation at 365 nm); δ ($CDCl_3$) 8.35—7.84 (3 H, m, Hs on pyridine ring), 7.10 (1 H, distorted s, 2-H on dihydronicotinamide ring), 7.04 and 6.95 (3 H, both s, Hs on benzene ring), 5.65 (1 H, d, 6-H on dihydronicotinamide ring), 5.15br (3 H, s, amide NH), 4.70 (1 H, m, 5-H on dihydronicotinamide ring), 4.29 (2 H, s, CH_2N; 1 H, quintet, *H*CNH), 3.17br (2 H, s, 4-H on dihydronicotinamide ring), 2.58 (4 H, distorted t, benzyl), and 2.0—1.0 (34 H, m, other methylene Hs); *m/e* 656 (M^+).

N-{10(11)-*Oxo*[20]*paracyclophan*-22-*ylmethyl*}-1,4-*dihydronicotinamide* (*PCP–HNA*).—This was obtained in 28% yield by essentially the same procedure as employed for the preparation of HNA–PCP–Py: $\lambda_{max.}$ [CH_2Cl_2–MeOH (100 : 1 v/v)] 354.5 nm (ε 5 600); δ (CD_3OD) 7.13br (4 H, s, 2-H on dihydronicotinamide ring and Hs on benzene ring), 5.8br (1 H, d, 6-H on dihydronicotinamide ring), *ca.* 4.8br (1 H, 5-H on dihydronicotinamide ring obscured by overlapping resonance of solvent OH), 4.39 (2 H, s, CH_2N), 3.16br (2 H, s, 4-H on dihydronicotinamide ring), 2.62 (4 H, distorted t, benzyl), 2.40 (4 H, m, CH_2 adjacent to C=O), and 2.0—1.0 (30 H, m, other methylene Hs); *m/e* 506 (M^+).

Both HNA–PCP–Py and PCP–HNA decomposed gradually in air at room temperature. Accordingly, they were stored under nitrogen at −20 °C.

Stability Constants.—Electronic spectra were recorded for a series of solutions containing a cyclophane species (PCP) at a fixed concentration and varying amounts of $ZnCl_2$ at 25.0 ± 0.1 °C. The concentration of a cyclophane–Zn^{II} complex [PCP–Zn^{II}] at a given concentration of $ZnCl_2$ was calculated by equation (6), where A_c and A_f stand for the absorbances in the presence of a sufficient excess of $ZnCl_2$ and in its absence, respectively, at a wavelength near the absorption maximum of PCP–Zn^{II}, ΔA is the absorbance change relative to A_f in the presence of $ZnCl_2$ at the intermediate concentration range, and $[PCP]_0$ refers to the total concentration of PCP. The stability constant (K_{11}) for the

$$[PCP\text{–}Zn^{II}] = \frac{\Delta A}{[A_c - A_f]}\,[PCP]_0 \qquad (6)$$

formation of a 1 : 1 (ligand : metal) complex was calculated as defined by equation (7), where $[Zn^{II}] = [Zn^{II}]_0 -$

$$K_{11} = [PCP\text{–}Zn^{II}]/[PCP][Zn^{II}] \qquad (7)$$

[PCP–Zn^{II}], $[Zn^{II}]_0$ being the total concentration of zinc(II) ion. When the co-ordination equilibria involve the form-

ation of both 2 : 1 and 1 : 1 complexes [equation (8)], the

$$2(\mathrm{PCP}) \underset{K_1}{\overset{Zn^{II}}{\rightleftharpoons}} (2\mathrm{PCP}\text{–}Zn^{II}) \underset{K_2}{\overset{Zn^{II}}{\rightleftharpoons}} 2(\mathrm{PCP}\text{–}Zn^{II}) \quad (8)$$

respective stability constants (K_1 and K_2) were calculated as follows. An apparent molar absorption coefficient per PCP unit for a 2 : 1 (ligand : metal) complex is assumed to be identical with one for the corresponding 1 : 1 complex. Let c and x be a total concentration of PCP bound to Zn^{II} and a molar concentration of a 2 : 1 complex, respectively; K_1 and K_2 are expressed by equations (9) and (10). The

$$K_1 = x/[\mathrm{PCP}]^2[Zn^{II}] \quad (9)$$

$$K_2 = (c - 2x)^2/x[Zn^{II}] \quad (10)$$

material balance for Zn^{II} is given by equation (11). Equations (9) and (10) convert into equations (12) and (13),

$$[Zn^{II}] = [Zn^{II}]_0 - x - (c - 2x) = [Zn^{II}]_0 + x - c \quad (11)$$

respectively, upon incorporation of equation (11). Combination of equations (12) and (13) and rearrangement

$$K_1 = x/[\mathrm{PCP}]^2([Zn^{II}]_0 + x - c) \quad (12)$$

$$K_2 = (c - 2x)^2/x([Zn^{II}]_0 + x - c) \quad (13)$$

result in equation (14). At a given concentration of $ZnCl_2$, c and hence [PCP] can be calculated by equation (6).

$$(K_1K_2)\ [\mathrm{PCP}]([Zn^{II}]_0 - c) + K_1[\mathrm{PCP}]^2(2[Zn^{II}]_0 - c) = c \quad (14)$$

Thus, equation (14) is in the form $aX + bY = Z$, where a and b are $(K_1K_2)^{\frac{1}{2}}$ and K_1, respectively, and X, Y, and Z are experimentally observable. K_1 and K_2 were determined by least-squares analysis using all the experimental sets of (X, Y, Z) at various concentrations of $ZnCl_2$. Conversely, for a set of given K_1 and K_2 values, equation (14) was solved for c at the zinc concentrations experimentally employed. The theoretical lines in Figure 2 were constructed in this manner.

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