Marine metabolites and metal ion chelation. Circular dichroism studies of metal binding to *Lissoclinum* cyclopeptides

David J. Freeman,[a] Gerald Pattenden,*[,a] Alex F. Drake*[,b] and Giuliano Siligardi[b]

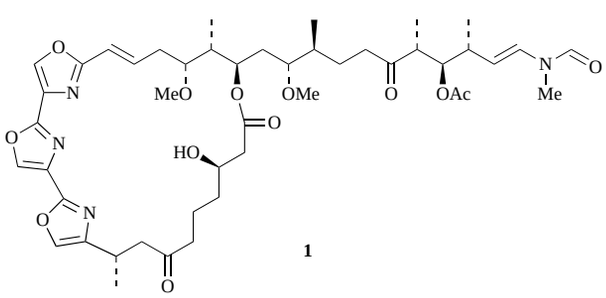
[a] *Department of Chemistry, Nottingham University, Nottingham, UK NG7 2RD*
[b] *Department of Pharmacy, Kings College London, Manresa Road, London, UK SW3 6LX*

Variable temperature circular dichroism (CD) spectra of the patellamides A (3), B (4) and E (5), isolated from the ascidian ('sea squirt') *Lissoclinum patella*, show that they have very similar thermodynamically preferred macrocyclic ring conformations. In addition, the CD profile of the 'figure eight like' conformation 10 in the patellamides has been defined, and CD spectroscopy is shown to provide an insight into the interconversions between the limiting conformations, 9 and 10, in this family of cyclopeptides. The cyclopeptides 3–5 bind both Cu^{2+} and Zn^{2+} and CD studies show that as a family, in line with previous studies, they can bind more than one metal ion per molecule. Thus, the first binding domain for the three patellamides shows a binding constant in the range 2×10^4 to 2×10^5, and a second binding site for patellamide B (4) has $K = 230$ (Cu^{2+}) and $K = 16$–20 (Zn^{2+}). The CD spectra of the patellamide–metal conjugates can be correlated with the 'square form' conformation 9 of the cyclopeptides. This best fit situation *vis-à-vis* metal chelation, could have important implications regarding the biological activity and *modus operandi* of the cyclopeptides *in vivo*.

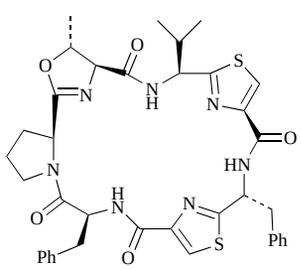
The marine environment is teeming with novel and unusual secondary metabolites, many of which have already shown considerable promise for development as therapeutic agents.[1] But to what purpose are these diverse and often structurally bizarre compounds produced *in vivo*? The seas and the oceans contain substantial amounts of dissolved inorganic salts. Furthermore, many marine natural products have structural features which make them ideal for interacting with metals, *e.g.* macrocyclic cavities, polar functional groups, in chelating arrangements with potential for wrapping around metal ions.[2] These features of our marine environment beg the questions: (*i*) Can/do marine metabolites sequester and transport metal ions? (*ii*) Do metals provide a template for biological assembly of the metabolites? (*iii*) Could metal–ligand complexation play a part in the pronounced biological activity of many of the compounds? It is with these questions in mind that a few years ago we embarked on a programme to study the syntheses and ionophoric properties[2] of members of the unusual oxazole- and thiazole-containing macrolides the ulapualides, *e.g.* **1**,[3] produced by nudibranchs and sponges, and the *Lissoclinum* cyclopeptides, *e.g.* lissoclinamide 5 (**2**), isolated from ascidians ('sea squirts').[4]

More than twenty five members of the *Lissoclinum* family of cyclopeptides have now been isolated from ascidians.[5] They vary in structural complexity according to: (*i*) their ring size, *i.e.* 18 to 24-membered hexa-, hepta- and octa-peptides; (*ii*) the nature of their amino acid residues; (*iii*) the presence, position and number of oxazole, thiazole, oxazoline and thiazoline rings making up their macrocycle cavities. Total synthesis has played a dominant role in establishing structure amongst these cyclopeptides,[6] and interesting and unusual metal-chelation properties have already been described for certain of their number.[7] We now describe our studies of the binding properties of the lissoclinum cyclopeptides, **3**, **4** and **5** (also known as 'patellamides' A, B and E respectively) with zinc(II) and copper(II) ions, using circular dichroism (CD) and mathematical modelling techniques. Contemporaneous complementary CD studies of lissoclinamide–metal conjugates and their biosynthetic congeners will be published separately.

The 'patellamide' family of cyclooctapeptides are characterised by the presence of two thiazole and two oxazoline rings



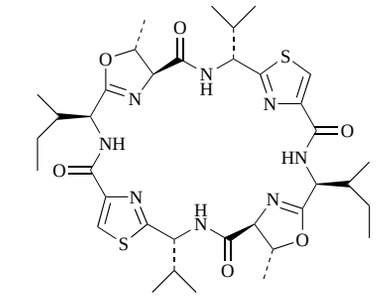
1



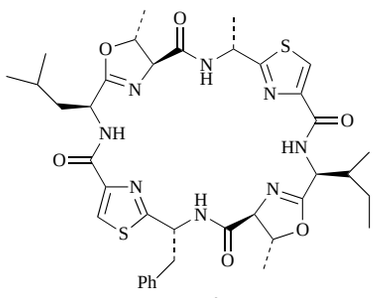
2

which form part of a conformationally restrained 24-azacrown-8 macrocyclic framework, *viz*. **3**–**5**.[8] The organism, *Lissoclinum patella*, from which they have been isolated, has been found to concentrate several metals, including copper, to ten thousand times the concentration found in the local marine environment.[9] Indeed, both mono- and di-nuclear copper complexes have been described following treatment of patellamide D **6** with $CuCl_2$–Et_3N, and the X-ray structure has been reported for a novel dinuclear copper complex, with a bridging carbonate anion, *viz*. **8**, of the related cyclooctapeptide ascidiacyclamide **7**.[10]

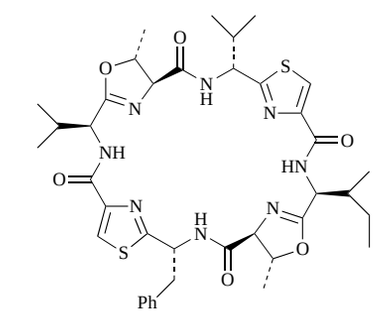
The aforementioned X-ray studies complement earlier investigations by Ishida *et al.*[11] and Schmitz *et al.*,[12] and more recent independent work by Ishida *et al.*[13] and by Fairlie *et al.*[14] using NMR spectroscopy, concerning the most likely conformations adopted by the patellamide natural products in solution. These investigations, taken together, have shown that



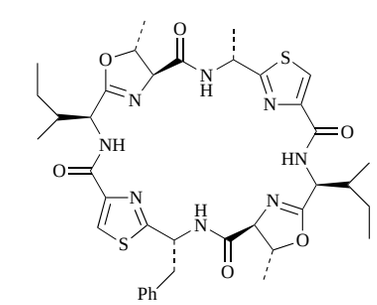
3



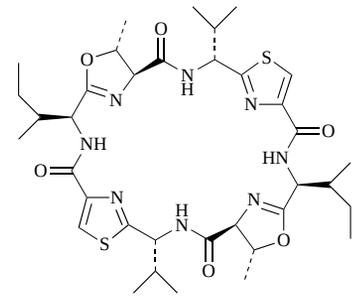
4



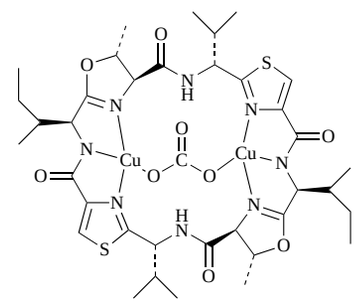
5



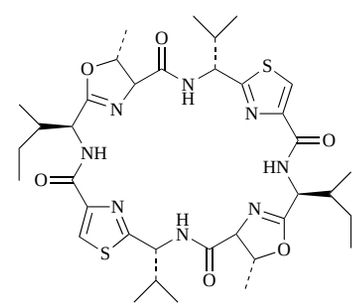
6



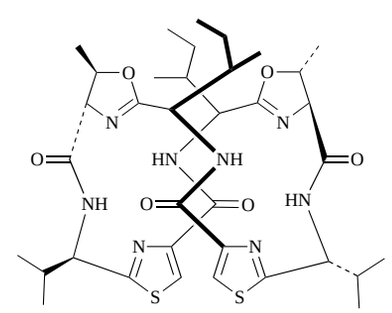
7



8



9



10

C_2-symmetric patellamides like ascidiacyclamide **7** and patellamide A **3** have predominantly a 'square form' conformation, *viz*. **9**, whereas the non C_2-symmetric patellamides B **4** and D **6** assume largely twisted 'figure eight like' conformations, *viz*. **10**. The preference for one or other of these conformations is clearly dependent on the nature and stereochemistry of the alkyl residues substituting the C_2-symmetric macrocyclic backbone in the patellamides. These observations are important for our discussions of the CD spectra of the patellamides **3**–**5** and their metal conjugates.

Results and discussion

Variable temperature CD and conformation of the patellamides 3–5 in methanol

The CD spectrum of a typical polypeptide derives from the spectroscopic interaction of an amide chromophore with its ordered neighbouring amides. Accordingly, a particular CD spectrum profile can be correlated with a particular oligopeptide conformation (α-helix, β-sheet, β-turn).[15] The CD spectra of patellamides A (**3**), B (**4**) and E (**5**) are illustrated in Fig. 1. The strong CD absorption at *ca.* 250 nm in these spectra cannot originate from a peptide amide bond and this feature precludes the conventional CD/peptide conformational analysis. The patellamides have a peptide-like core containing oxazoline and thiazole rings. The observed CD of the patellamides must, therefore, originate from spectroscopic interactions between electronic excitations based on the heterocyclic rings, the thiazoles in particular. The CD spectra will be sensitive to the relative orientations of these heterocyclic rings as a consequence of π–π stacking interactions (referred to by Ishida *et al.*[13]) with only minor spectroscopic contributions from the amino acid side chains and the amide groups.

Linear oligopeptides are known for their flexibility when free, unbound in aqueous solution.[15] Ordered conformations are adopted on binding to the relevant active site, in non-aqueous environments or at low temperatures. Higher temperatures see oligopeptides in a dynamic state of populated local energy minima. Lower temperatures see the oligopeptide frozen into a

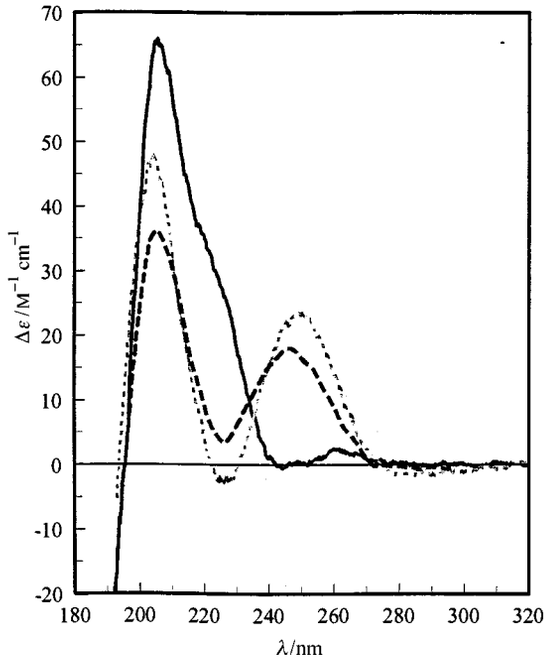


Fig. 1 Room temperature CD spectra in methanol: (——) patellamide A, **3**; (----) patellamide B, **4**; and (– – –) patellamide E, **5**

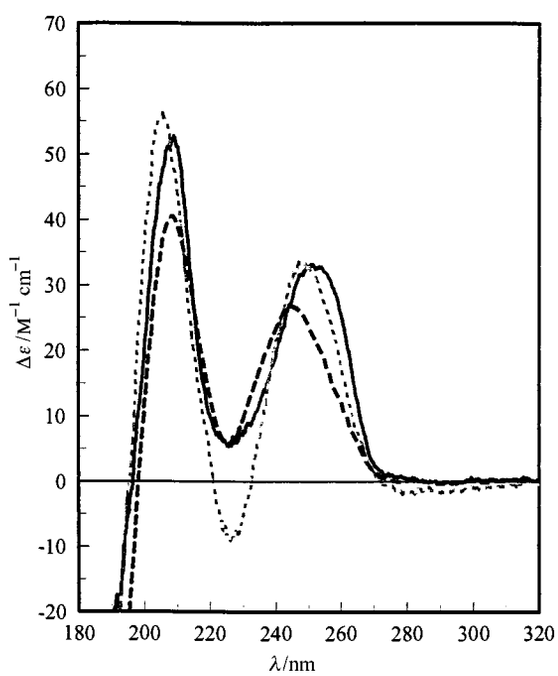


Fig. 2 Low temperature CD spectra in methanol: (——) patellamide A, **3**; (----) patellamide B, **4**; and (– – –) patellamide E, **5**

single, thermodynamically preferred conformation. The same cautionary note applies to cyclic peptides. The 24-membered macrocyclic ring in the patellamides **3**–**5** is likely to present a time-averaged conformation, difficult to characterise by NMR spectroscopy.[1] A CD study involving temperature dependence is therefore an important prelude to a detailed conformation analysis and will help in discriminating contributions from different conformation types. Fig. 2 illustrates the CD spectra of patellamides A, B and E at low temperature in methanol demonstrating that at low temperatures the three patellamides adopt similar thermodynamically preferred conformations.

The CD spectra of the non C_2-symmetric patellamides B (**4**) and E (**5**) are relatively similar at all temperatures, with characteristic prominent positive CD peaks at *ca.* 250 and 204 nm. In both cases, there is a relatively small CD/temperature effect,

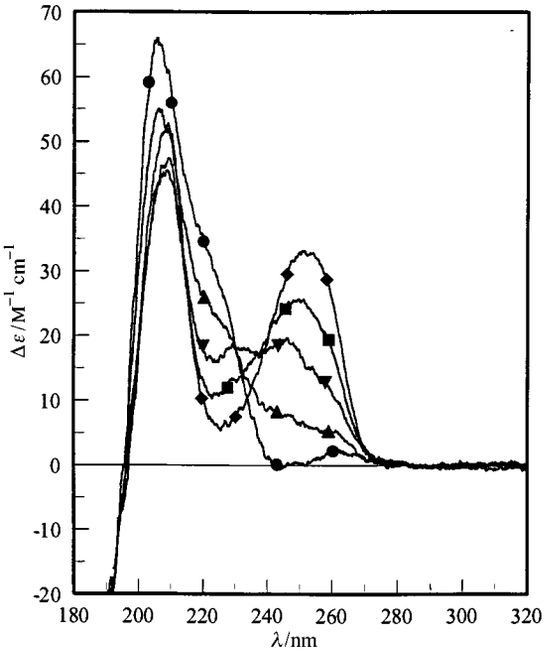


Fig. 3 Variable temperature CD spectra of patellamide A in methanol at temperatures: (◆) 74; (■) −62; (▼) −45; (▲) −4; (●) 26 °C

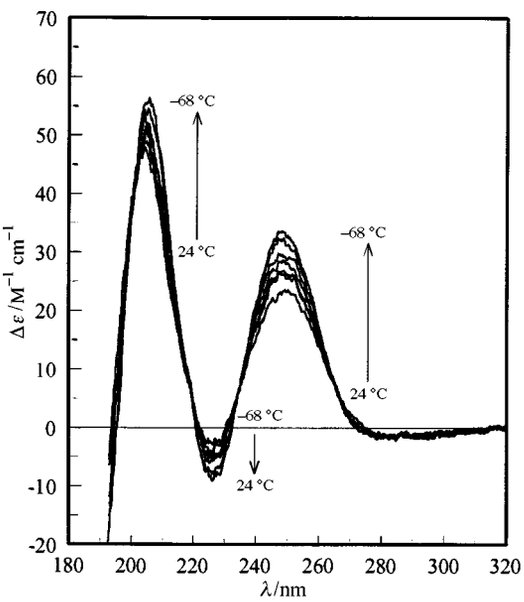


Fig. 4 Variable temperature CD spectra of patellamide B in methanol

with isosbestic points, consistent with a single, well populated global minimum energy state, fully populated towards −100 °C (Figs. 4–6). The work of Ishida *et al.*[13] enables the correlation of the low temperature CD spectra of the patellamides presented in Figs. 4 and 5 with the 'figure eight like' conformation **10** with conformational flexibility (a dynamic state) being registered at and above ambient temperatures.

The CD behaviour of C_2-symmetric patellamide A (**3**) is more complex (Figs. 1, 3 and 6). The replacement of the phenyl residue in patellamides B and E with a valine residue is critical. This is not a spectroscopic effect, as demonstrated by the variable temperature CD (Fig. 3). On cooling, evidence is clearly presented for three conformational states for patellamide A with an isosbestic point at 229 nm connecting the two higher temperature states. This isobestic point is lost as the final low temperature state becomes fully populated. At *ca.* −50 °C an equilibrium of populated local energy minima is reached (Fig. 6). Below −100 °C patellamide A is frozen in the global energy minimum, the 'figure eight like' conformation **10** similar to

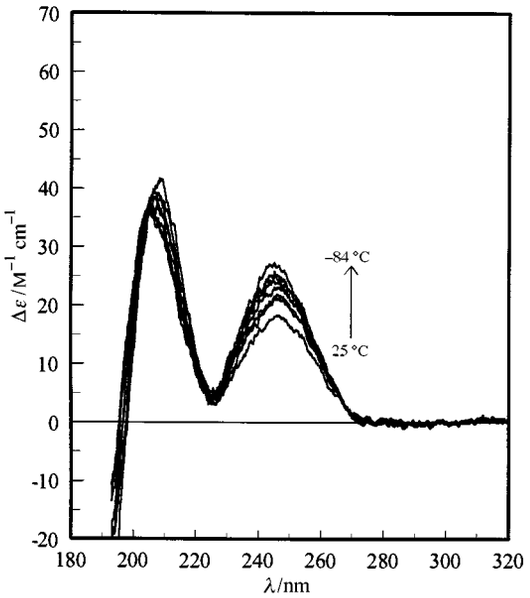


Fig. 5 Variable temperature CD spectra of patellamide E in methanol

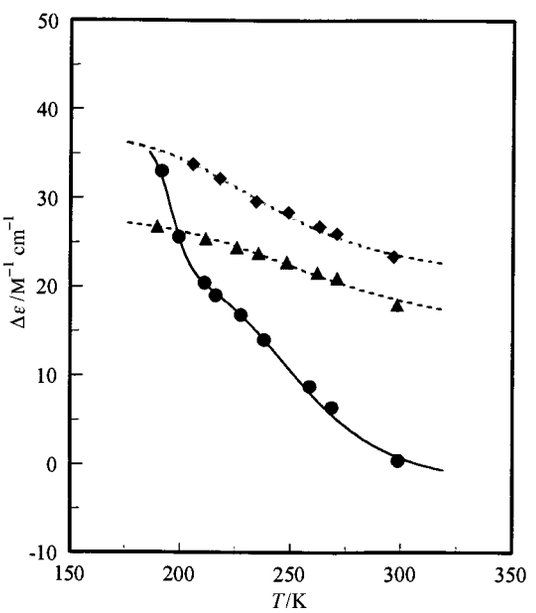


Fig. 6 Plot of CD maximum near 250 nm *versus* temperature: ◆ patellamide B; ▲ patellamide E; ● patellamide A

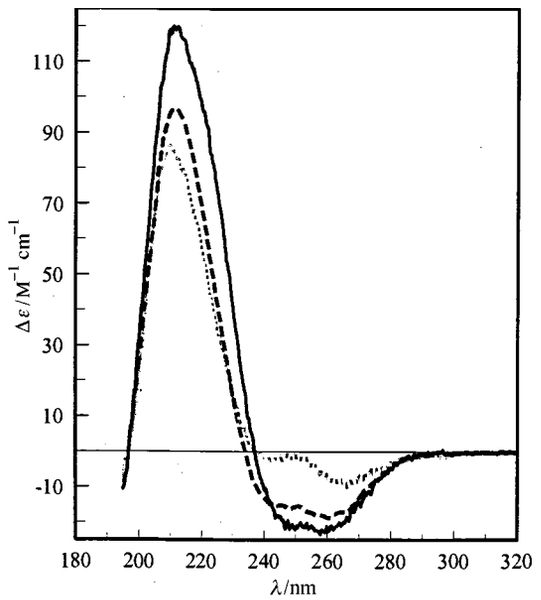


Fig. 7 CD spectra of Cu^{2+} bound patellamides in methanol: (——) patellamide A, **3**; (----) patellamide B, **4**; and (– – –) patellamide E, **5**

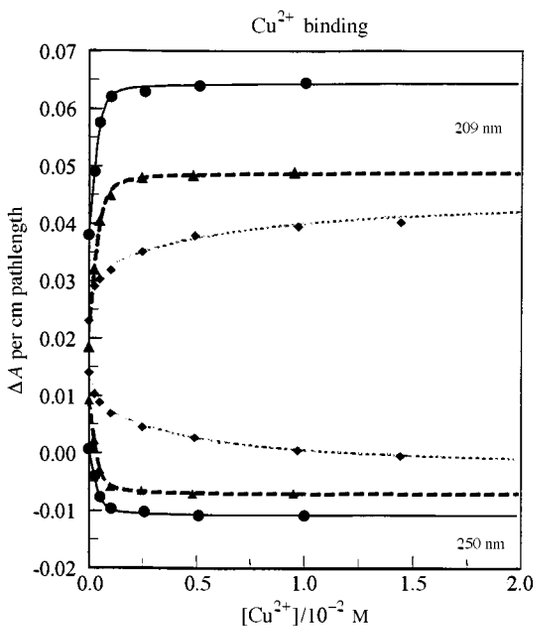


Fig. 8 CD/Cu^{2+} titration data and fitted curves: (——) patellamide A, **3**; (----) patellamide B, **4**; and (– – –) patellamide E, **5**

patellamides B and E (Fig. 2). The CD signature for the alternative 'square form' conformation **9** must, therefore, contribute to the CD spectra of patellamide A at ambient temperatures. The high temperature dynamic state of patellamide A is different to that of patellamides B and E. At ambient temperatures the contribution of the 'square form' state **9** in patellamide A is more significant. These data therefore complement the X-ray and NMR studies described earlier.[11–14] NMR studies of flexible molecules are difficult due to time-scale averaging of conformational mobility. The conformational analysis presented here clarifies the uncertainties encountered by Ishida *et al.*[13] when analysing the NMR data for patellamide A.

CD and the binding of metal ions in the patellamides 3–5

In the present study we examined the binding of the patellamides **3**–**5** to the chloride salts of magnesium, calcium, copper and zinc in methanol as solvent. The salt solutions were titrated separately into methanol solutions of the three cyclopeptides, and their CD spectra, between 190–320 nm, were then recorded. Although Cu^{2+} and Zn^{2+} were both found to bind the patellamides, we found no evidence for Mg^{2+} or Ca^{2+} binding. Fig. 7 presents an overlay of the CD spectra of the limit bound Cu^{2+} (patellamide ⩾20:1). Similar CD limit spectra are presented in Fig. 9 for the Zn^{2+} complexes. A major difference in the electronic spectroscopy of Cu^{2+} and Zn^{2+} in the 220–280 nm region is the presence of a ligand↔metal charge transfer transition in the case of Cu^{2+}. The presence of the 260–270 nm negative peak or shoulder in the Cu^{2+} spectra, absent in the Zn^{2+} complexes, can be assigned as the charge transfer, and this is confirmed by the ordinary UV absorption spectrum (not shown). Zn^{2+} is relatively transparent in the 220–280 nm region and the CD of the Zn complex in this region is, therefore, ligand based. The negative CD of the complexes at *ca.* 250 nm can be correlated with the 'square form' conformation **9** as inferred in the higher temperature CD spectra of patellamide A. The shape of the Zn–patellamide spectra is of a type necessary to compensate for the 'figure eight like' CD component to give the near zero CD observed at *ca.* 250 nm for patellamide A at ambient temperature (Fig. 1). This is also in accord with the report of Ishida *et al.* that, although the patell-

Table 1 Patellamide–metal binding parameters

Metal	Peptide	λ/nm	K_1[a]	K_2[a]	$\Delta\varepsilon_0$[b]	$\Delta\varepsilon_1$[b]	$\Delta\varepsilon_2$[b]
Cu	Patellamide A	210	2×10^4	—	71.00	120.50	—
		250	2×10^4	—	1.3	−20.17	—
	Patellamide B	210	3×10^5	230	45.76	63.50	96.00
		250	3×10^5	230	27.70	16.00	−5.80
	Patellamide E	210	1.5×10^4	—	36.70	97.30	—
		250	1.5×10^4	—	18.26	−13.9	—
Zn	Patellamide A	210	3×10^4	16	73.00	79.00	130.00
		250	3×10^4	16	0.86	−2.00	−26.10
	Patellamide B	210	3×10^4	18	46.90	51.00	82.00
		250	3×10^4	20	27.80	24.90	−18.20
	Patellamide E	210	8×10^4	20	37.70	52.00	130.00
		250	8×10^4	20	17.40	10.50	−32.00

[a] K_1 and K_2 represent the binding constants for the first and second Cu sites respectively. [b] $\Delta\varepsilon_0$, $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ represent the limiting $\Delta\varepsilon$s of the respective sites at the relevant wavelengths.

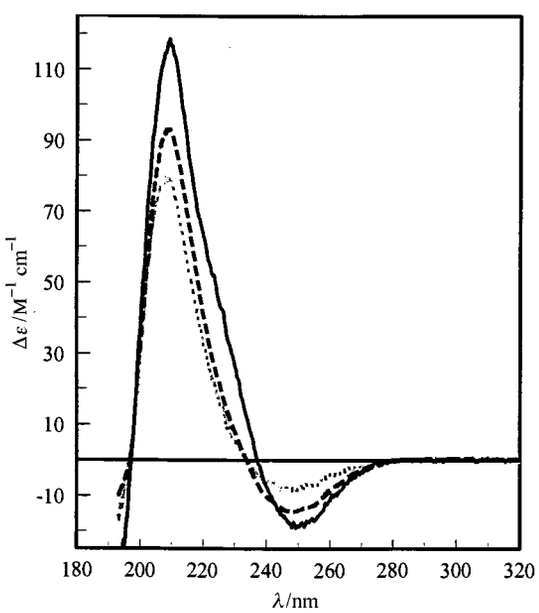


Fig. 9 CD spectra of Zn^{2+} bound patellamides in methanol: (——) patellamide A, **3**; (- - - -) patellamide B, **4**; and (– – –) patellamide E, **5**

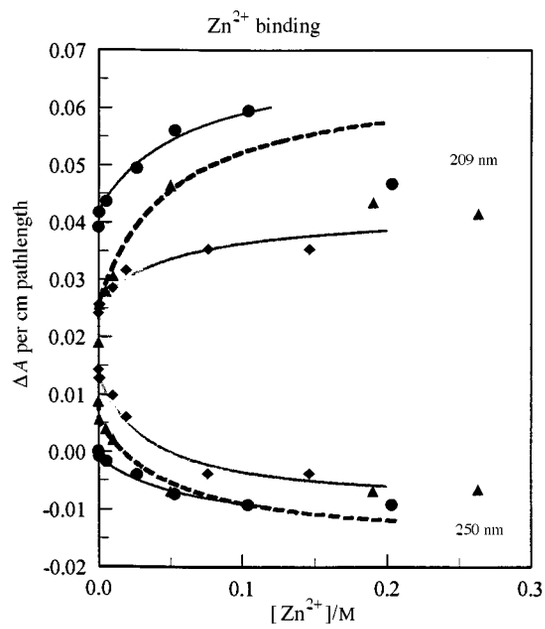


Fig. 10 CD/Zn^{2+} titration data and fitted curves: (——) patellamide A, **3**; (- - - -) patellamide B, **4**; and (– – –) patellamide E, **5**

amides may exist in the 'figure eight like' conformation **10** in free solution, it is the square form conformation **9** that is adopted to accommodate biological activity and the trapping of water and ethanol.

Assessing the ability of a ligand to bind a metal is normally described in terms of a binding constant. Titrations of a fixed concentration of each of the patellamides **3**–**5** with varying concentrations of metal ion see changes in the CD spectra from those represented in Fig. 1 to those represented in Figs. 7 and 9. In all cases isosbestic points were observed indicating two state systems between bound and unbound metal ions. However, the titration curves, monitored at wavelengths 250 and 209 nm, showed discontinuities (Figs. 8 and 10). In line with previous studies, we conclude from these data that the patellamides, as a family, can bind more than one metal ion per molecule. Fitting the equation described in the Experimental section, the binding constants listed in Table 1 were obtained.

The CD spectra of Cu(patellamide A) and Cu(patellamide E) are similar, with the titration curve adequately reproduced by a 1:1 Cu–patellamide complex (Figs. 7 and 8). The titration curve of patellamide B with Cu^{2+} needs to be fitted with two binding constants. The first Cu^{2+} is bound more strongly by patellamide B than for either patellamide A or E. The second Cu^{2+} site is relatively weak in patellamide B and not apparent in patellamide A or patellamide E. Figs. 7 and 8 confirm that the conformation of the final metal–ligand bound state in patellamide B is significantly different from patellamide A and patellamide E. The presence of the alanine residue in patellamide B, in place of the valine residue in patellamides A and E clearly plays an important role.

The binding of zinc to the patellamides **3**–**5** is not simple, although they bind Zn^{2+} ions with similar abilities. Three binding regimes can be clearly identified (Figs. 10 and 11). The first two regimes, below a metal–ligand ratio of 100:1, require a two metal binding model. Above a 100:1 Zn–patellamide ratio there is a strong decrease in CD intensity as the third zinc ion is accommodated. Only data points (no curves) are presented for the very high zinc containing solutions.

Note added in proof

CD spectroscopy is extremely sensitive to the environment of the absorbing group (patellamide chromophore). Accordingly, the approximations often taken in conventional approaches (*e.g.* to linearise the formulae) are not appropriate. The full description of a two binding site model requires knowledge of four binding constants and the limiting CD values of four

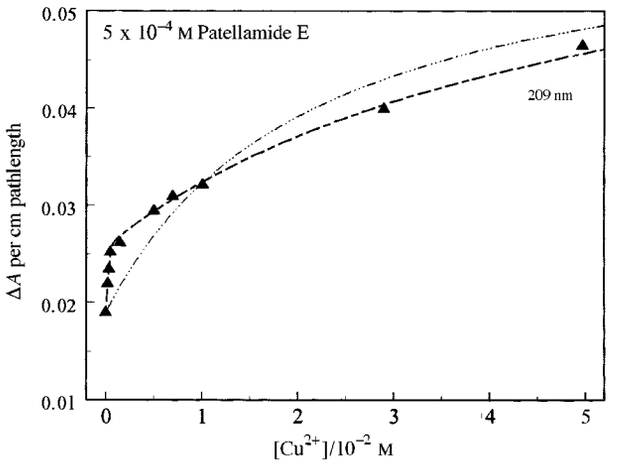


Fig. 11 Expanded representation of Zn^{2+}/patellamide E titration: (– – –), two binding modes (see Table 1); (· — ··· — ·), one binding mode ($K = 48$)

species. The approximation made in the present work is that the binding is progressive with only two binding constants. The CD of the zero bound species is taken from the solution of the free patellamide (no metal); the CD of the two bound metal ions is taken from the limiting CD at high metal ion concentrations; the CD of the single metal ion species comes from fitting the data. The data presented in Table 1 are those which provide the curves drawn in Figs. 8 and 10. The titration curve for the Zn^{2+}/patellamide E titration, monitored at 209 nm, is expanded in Fig. 11 as an example. Also shown is an example of a good-fit single binding site curve $K = 48$. Of importance is the *discontinuity* in the experimental data at about a 1 : 1 metal ion–patellamide ratio.

Table 1 reveals that the first binding domain of the patellamides **3**–**5** with Cu^{2+} and Zn^{2+} are covered by a binding constant in the range of 2×10^4 to 3×10^5. A second binding site has been identified for patellamide B/Cu^{2+} with $K = 230$, and for the patellamides A, B and E with Zn^{2+}, $K = 16$–20.

Conclusions

The patellamide family of cyclooctapeptides incorporates alkyl (amino acid-derived) side-chains which are critical in controlling their molecular conformation, metal binding and biological activity.

Although not apparent at room temperature, the thermodynamically preferred macrocyclic ring conformations of the patellamides A (**3**), B (**4**) and E (**5**) are all very similar. In some senses this is contrary to the conclusion reached by Ishida *et al.* from NMR studies. However, the 24-membered macrocyclic ring of the patellamides is not conformationally rigid and room temperature studies must be treated with caution.

The dependence of ring conformation on alkyl group substituent in the patellamides **3**–**5** has important consequences for both their ease of synthesis and ionophoric properties. CD spectroscopy provides a straightforward method of assigning conformation to members of the patellamide family and, importantly, insight into the conversion between their limiting conformations **9** and **10**. In particular, the CD profile of the 'figure eight like' conformation **10** has been defined. An important role for the phenylalanine residue (*e.g.* in patellamide E) in stabilising molecular conformation has been identified.

Equally, our study has shown that CD is an important tool for the determination of metal binding constants uncomplicated by paramagnetism or free metal contributions and providing insight into multi-binding characteristics. In the present case, an alanine residue has been seen to be important for controlling Cu^{2+} binding to patellamide B (**4**).

The correlation between CD spectroscopy and cytotoxicity will hopefully contribute to providing a rationale for a molecular conformation–biological activity relationship in the *Lissoclinum* family of cyclopeptides.

Experimental

Patellamides A, B and E (3–5)

The patellamides were isolated from *Lissoclinum patella* by C. M. Ireland and his colleagues. Their characterisations and spectroscopic data have been published previously.[8]

CD spectroscopy

CD spectra were measured with a Jasco J720 Spectropolarimeter complemented with ordinary UV absorption measurements employing an AVIV 17DS spectrophotometer. Measurements were made with a peptide concentration of typically 5×10^{-4} M in spectroscopic grade methanol (BDH) employing a 0.2 mm pathlength cuvette. Low temperature measurements were performed as described previously.[16] Titrations were performed by adding the appropriate quantities of methanol solutions of analar grade metal chlorides (BDH) to the 5×10^{-4} M methanol solution of the peptide.

Data analysis

Variable temperature CD spectra. Computer fits to the variable temperature CD spectra were generated based on Van't Hoff plots derived from expression (1), where K is the

$$\frac{\mathrm{d}(\log K)}{\mathrm{d}T} = \frac{\Delta H}{RT} \quad (1)$$

equilibrium constant between two conformational states and T is the solution temperature in K.

A two component equilibrium requires two Van't Hoff terms based on the equilibrium:

$$\text{Ordered State 2} \rightleftharpoons \text{Ordered State 1} \rightleftharpoons \text{Dynamic State}$$

giving eqn. (2), where ΔA_{obs} is the observed CD of the test solution

$$\Delta A_{obs} = \Delta A_d + \Delta A_1 + \Delta A_2 \quad (2)$$

and ΔA_d, ΔA_1 and ΔA_2 are the CD contributions of respectively the dynamic state, the ordered state 1 and the ordered state 2.

The application of Beer's law gives, for example, eqn. (3),

$$\Delta A_d = \Delta\varepsilon_d[\text{Dynamic state}]l \quad (3)$$

where $\Delta\varepsilon_d$ is the differential molar extinction coefficient of the dynamic state, [Dynamic state] is the concentration of the dynamic state and l is the cuvette pathlength. The differential molar extinction coefficients $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ can be similarly defined. For the two temperature equilibria, enthalpies ΔH_1 and ΔH_2 and mid-temperatures (melting temperatures) T_{m1} and T_{m2} can be defined. Substitution leads to expression (4) for the observed CD at any temperature T.

$$\Delta\varepsilon_{obs} = \left[\frac{\Delta\varepsilon_d \exp\left(-\frac{\Delta H_1}{RT} + \frac{\Delta H_1}{RT_{m1}}\right) + \Delta\varepsilon_1}{1 + \exp\left(-\frac{\Delta H_1}{RT} + \frac{\Delta H_1}{RT_{ml}}\right)}\right] + \left[\frac{\Delta\varepsilon_1 \exp\left(-\frac{\Delta H_2}{RT} + \frac{\Delta H_2}{RT_{m2}}\right)}{1 + \exp\left(-\frac{\Delta H_1}{RT}\right) + \left(\frac{\Delta H_1}{RT_{m2}}\right)}\right] + \Delta\varepsilon_1 \quad (4)$$

The data in Fig. 6 were fitted to this equation.

CD and metal binding

Chloride salt solutions of copper and zinc were titrated separately into methanol solutions of the patellamides **3**–**5**, and their CD spectra, between 190–320 nm, were then recorded. A single metal binding is described by eqn. (5), with the binding constant given by eqn. (6).

$$M^{2+} + \text{ligand} = [M(\text{ligand})]^{2+} \quad (5)$$

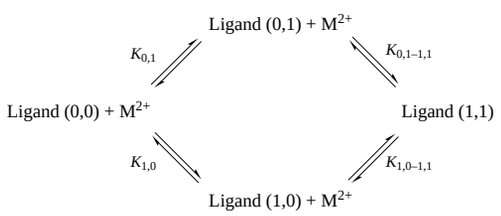
$$K = \frac{[M^{2+}][\text{Ligand}]}{[M(\text{ligand})]} \quad (6)$$

At equilibrium, the CD at any wavelength is given with the knowledge that the free metal has no CD by eqn. (7).

$$\Delta A_{\text{obs}} = \Delta A_{\text{ligand}} + \Delta A_{\text{M(ligand)}} \quad (7)$$

Based on Beer's Law, the concentrations of the individual species can be determined and the value of K calculated.

The binding of two metal ions to a single ligand is given by Scheme 1, where the parentheses and subscripts refer to the



Scheme 1

occupation of the relevant metal binding site *e.g.* (0,0) indicates both sites unoccupied, (1,1) indicates both sites are occupied.

At equilibrium, there are four binding constants which need to be determined, requiring knowledge of four differential extinction coefficients and the concentration of four species. The mathematical analysis of this situation is complex requiring computational methods.[17] This was achieved in the present case employing a Mathsoft Mathcad computer program with the assumption that the metal binding is progressive with $K_{1,0}$ and $K_{1,0-1,1}$ set to zero.

The measurement *errors* in the data are clear from the spectra presented—internally to this series of measurements the precision is good. This will be negligible compared to the data analysis errors. The data analysis, for the chosen model, was undertaken employing an in-house implementation of the Marquardt–Levenberg non-linear least squares algorithm.[17] The model was restricted to 1-, or 2-metal binding as appropriate. Each data point was given equal weighting and the curves produced were at the 95% confidence limit. In Fig. 10, the data fitting is very good up to Zn^{2+} concentrations of the order 0.1 M Zn (200 : 1, Zn : patellamide). Above a 100 : 1 ratio there is progressive evidence for a third binding mode. The concentration of the latter will be relatively low in the good-fit region where it has been ignored for the analysis.

Acknowledgements

We thank Professor C. M. Ireland for providing samples of patellamides A, B and E, and The Lawes Trust Institute for a research scholarship (to D. J. F.). We also thank the EPSRC for provision of CD instrumentation and facilities, and Dr A. J. Potter and Mr J. Rutt for some preliminary experiments, synthetic work and discussions.

References

1 See: D. J. Faulkner, *Nat. Prod. Rep.*, 1996, **13**, 75, and earlier reviews in this series. See also: D. J. Faulkner, *Chem. Rev.*, 1993, **93**, 1671.
2 J. P. Michael and G. Pattenden, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1.
3 See: S. K. Chattopadhyay and G. Pattenden, *Tetrahedron Lett.*, 1995, **36**, 5271; J. Maddock, G. Pattenden and P. G. Wright, *Computer-Aided Molecular Design*, 1993, **7**, 573, and references cited therein.
4 C. D. J. Boden and G. Pattenden, *Tetrahedron Lett.*, 1994, **35**, 8271; C. D. J. Boden and G. Pattenden, *Tetrahedron Lett.*, 1995, **36**, 6153. See also M. Norley and G. Pattenden, *Tetrahedron Lett.*, 1996, **37**, 9111, and references therein; P. Wipf and P. C. Fritch, *J. Am. Chem. Soc.*, 1996, **118**, 12 358.
5 For example: *Lissoclinamides*: B. M. Degnan, C. J. Hawkins, M. F. Lavin, E. J. McCaffrey, D. L. Parry, A. L. van den Brenk and D. J. Watters, *J. Med. Chem.*, 1989, **32**, 1349; C. J. Hawkins, M. F. Lavin, K. A. Marshall, A. L. van den Brenk and D. J. Watters, *J. Med. Chem.*, 1990, **33**, 1632. See also ref. 12 below. *Tawicyclamides A and B*: L. A. McDonald, M. P. Foster, D. R. Phillips, C. M. Ireland, A. Y. Lee and J. Clardy, *J. Org. Chem.*, 1992, **57**, 4616. *Patellins*: T. M. Zabriskie, M. P. Foster, T. J. Stout, J. Clardy and C. M. Ireland, *J. Am. Chem. Soc.*, 1990, **112**, 8080. For a review of metabolites from *L. patella* and other ascidians see; B. S. Davidson, *Chem. Rev.*, 1993, **93**, 1771.
6 For a recent review see: P. Wipf, *Chem. Rev.*, 1995, **59**, 2115. See also: Y. Hamada, M. Shibata and T. Shioiri, *Tetrahedron Lett.*, 1985, **26**, 5159; Y. Hamada, M. Shibata and T. Shioiri, *Tetrahedron Lett.*, 1985, **26**, 6501; U. Schmidt and H. Griesser, *Tetrahedron Lett.*, 1986, **27**, 163; Y. Hamada, S. Kato and T. Shioiri, *Tetrahedron Lett.*, 1986, **26**, 3223, and references under ref. 4 above.
7 For example: P. Wipf, S. Venkatraman, C. P. Miller and S. J. Geib, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1516; see also refs. 9 and 10.
8 C. M. Ireland, A. R. Durso, Jr., R. A. Newman and M. P. Hacker, *J. Org. Chem.*, 1982, **47**, 1807; J. E. Biskupiak and C. M. Ireland, *J. Org. Chem.*, 1983, **48**, 2302; L. A. McDonald and C. M. Ireland, *J. Nat. Prod.*, 1992, **55**, 376.
9 A. L. van den Brenk, D. P. Fairlie, G. R. Hanson, L. R. Gahan, C. J. Hawkins and A. Jones, *Inorg. Chem.*, 1994, **33**, 2280.
10 A. L. van den Brenk, K. A. Byriel, D. P. Fairlie, L. R. Gahan, G. R. Hanson, C. J. Hawkins, A. Jones, C. H. L. Kennard, B. Moubaraki and K. S. Murray, *Inorg. Chem.*, 1994, **33**, 3549.
11 Y. In, M. Doi, M. Inoue, T. Ishida, Y. Hamada and T. Shioiri, *Chem. Pharm. Bull.*, 1993, **41**, 1686.
12 F. J. Schmitz, M. B. Ksebati, J. S. Chang, J. L. Wang, M. B. Hossain and D. J. van den Helm, *J. Org. Chem.*, 1989, **54**, 3463.
13 T. Ishida, Y. In, F. Shinozaki, M. Doi, D. Yamamoto, Y. Hamada, T. Shioiri, M. Kamigauchi and M. Sugiura, *J. Org. Chem.*, 1995, **60**, 3944.
14 G. Abbenante, D. P. Fairlie, L. R. Gahan, G. R. Hanson, G. K. Pierens and A. L. van den Brenk, *J. Am. Chem. Soc.*, 1996, **118**, 10 384.
15 G. Siligardi and A. F. Drake: The importance of extended conformations and in particular, the P_{II} conformation for the molecular recognition of peptides: *Biopolymers* (*Peptide Science*) 1995, **37**, 281.
16 G. Siligardi, A. F. Drake, P. Mascagni, D. Rowlands, F. Brown and W. Gibbons, *Eur. J. Biochem.*, 1991, **199**, 545; *Int. J. Peptide Protein Res.*, 1991, **38**, 519.
17 (*a*) D. W. Marquarat, *J. Soc. Ind. Appl. Maths.*, *2*, 1963, 431; (*b*) A. F. Drake, unpublished work.

Paper 7/03530F
Received 21*st May* 1997
Accepted 4*th September* 1997