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Stereochemistry of ulapualides, a new family of *tris*-oxazole-containing macrolide ionophores from marine nudibranchs. A molecular mechanics study

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SUMMARY

A molecular mechanics study of the marine metabolite ulapualide A, which is suggested to have ionophoric properties, has been carried out on various metal chelated complexes in order to predict the stereochemistry of the natural product. The results suggest a stereochemistry for ulapualide A which is closely similar to structurally related marine metabolites, whose stereochemistries have been established by X-ray crystallography and by partial synthesis.

INTRODUCTION

The ulapualides, e.g. ulapualide A (**1**), are an extraordinarily novel family of *tris*-oxazole containing macrolides which were first isolated from the egg masses of the marine nudibranch *Hexabranchus sanguineus* [1]. Similar, structurally related macrolides, which differ only according to alternations in oxidation pattern and methyl group substitution along the aliphatic backbone, have been isolated from other nudibranchs and also from marine sponges; these metabolites have been variously called kabiramides [2,3], halichondramides [4,5], e.g. **2**, and mycalamides [6,7]. This unusual family of marine metabolites shows a wide variety of biological activities, including antifungal, anti-leukaemic and ichthyotoxic properties.

The ulapualides and relatives have structures based on a macrocyclic cavity incorporating nitrogen and oxygen ligands, and a side chain containing several oxy-donor atoms in chelating arrangements. It is one of our suppositions that the biological profile of the ulapualides is in part associated with their capacity to chelate and sequester metals, i.e. behave as ionophores [8]. Interestingly, although the gross structures of the ulapualides are secure, we have no knowledge of the relative stereochemistries of the several chiral centres the structures accommodate. In

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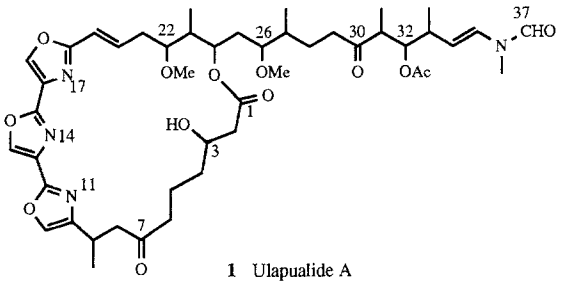
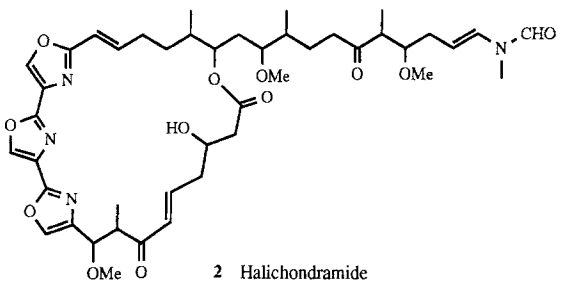


1 Ulapualide A

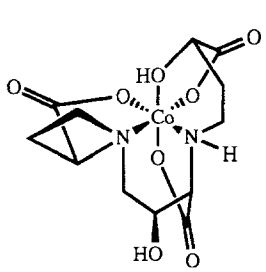


2 Halichondramide

tandem with our synthetic work amongst ulapualides and halichondramides [9a,b], we have carried out a molecular mechanics study of metal ion–ulapualide complexes in order to predict the most likely relative stereochemistry for this family of natural products. This study is summarised in this paper.

METHODS

All calculations were performed using the molecular-modelling software MACROMODEL [10] on a Silicon Graphics Personal IRIS workstation. The standard MM2 force field [11] was employed with additional parameters for Co(III) (see later) taken from a variety of sources (Table 1) [12–14]. These new parameters were first tested on two complexes [viz. [Co^{III}(mugineic acid)] (**3**) and [Co^{III}(*N*,*N*''-bis(salicylidene)-dipropylenetriamine)(1-methylimidazole)] (**4**)] for which X-



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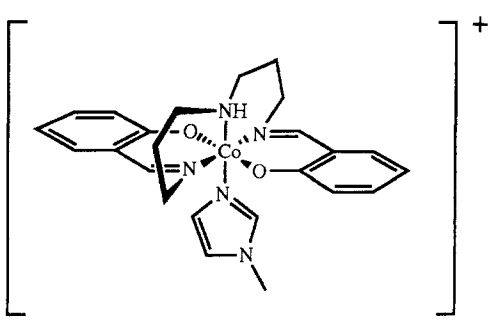

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TABLE 1
FORCE-FIELD PARAMETERS USED FOR THE Co^{3+} MODEL

Stretching force constants

Bond type	Bond length (Å)	k (mdyn $Å^{-1}$)	Bond moment (Debye)
Co–O (carbonyl)	1.9	2.5	2.5
Co–O (ether)	1.9	2.2	2.0
Co–N	1.9	2.0	2.0

Bending force constants

Angle type	Angle (°)	k (mdyn rad^{-2})
X–Co–X	90.0	0.220
C=O–Co	120.0	0.220
C–O–Co	109.6	0.220
C–N–Co	120.0	0.220

Torsional force constants (all zero except for those shown below)

Torsion bond	V1	V2	V3 (kcal mol^{-1})
Co–O=C–C	0.0	8.0	0.0
Co–N=C–C	0.0	8.0	0.0
Co–O–C–C	3.5	2.3	−3.5

Non-bonded interactions for Co(III)

Radius (Å)	ε (kcal mol^{-1})	Charge
3.00	0.224	+3

ray data were available [15,16], and good agreement between predicted and solid-state structures was observed (Fig. 1). Some initial calculations [17] were performed using a 'dummy' metal centre, and they utilised essentially the same parameters as given in Table 1, but the strain-free metal-ligand bond length was 2.1 Å, and the 'metal' centre had zero size (van der Waals radius) and no charge.

All minimisations were performed using the BATCHMIN routine and the Polak-Ribière minimisation method [18] to give a convergence of less than 0.01 kJ $Å^{-1}$. Starting geometries for conformational searches were generated using a random Monte-Carlo method to give between 500 and 1000 starting conformers. Ring closure bonds were allowed to vary between 1.0 and 3.0 Å, and all double bonds and stereocentres were constrained so as to maintain their geometry during the search. In practice, the metal–ulapualide complexes examined were found to be quite rigid conformationally, and as a consequence we were reasonably confident that global minima were located for the complexes using the criteria outlined. This constrasts sharply with the free

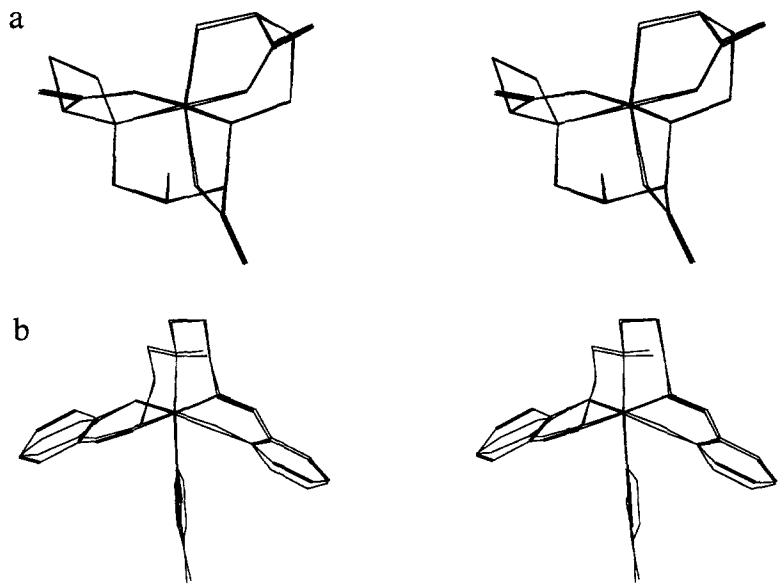


Fig. 1. Overlays of energy-minimised and X-ray crystal structures for (a) [Co^{III}(mugineic acid)], (b) [Co^{III} (*N*,*N*''-bis(salicylidene)dipropylenetriamine)(1-methylimidazole)].

ligand, which is highly mobile conformationally, and a corresponding global minimum could not be located with any degree of confidence.

RESULTS AND DISCUSSION

Preliminary examination of the 3D structure of ulapualide A (**1**) showed a macrocyclic cavity in which the aza-nitrogen atoms pointed towards the cavity centre, and the oxy atoms of the carbonyl and hydroxyl groups of the ring were also found to be freely accessible for metal binding. The side chain was found to be very flexible and could wrap over a metal centre either above or below the macrocycle to 'cap' a complex.

The donor atoms available in ulapualide A for complex formation are both numerous and mixed in type. The oxazole aza-nitrogen centres are expected to be 'soft' donors, and in common

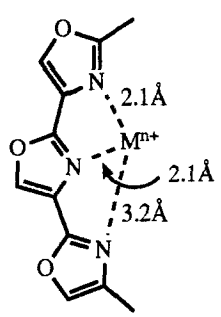
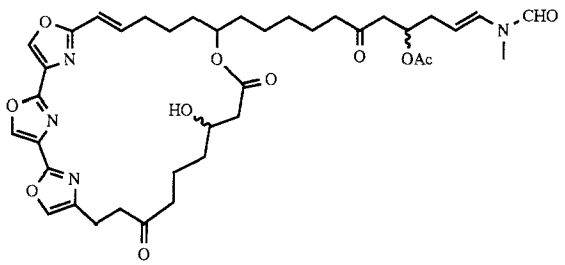


Fig. 2. The *tris*-oxazole metal complex.



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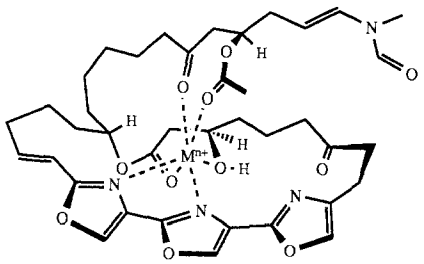

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with other heterocyclic aza donors they are likely to show selectivity for transition metal ion sequestration. The remaining carbonyl, ether and amide oxy atom donors are more typical of the familiar terrestrial ionophores, which are known to express a preference for alkali and alkali-earth metals. In view of the central role that the oxazole rings in ulapualide A must play in any encapsulation of a metal ion by the macrocyclic cavity, we chose to concentrate on the chelation of transition metals, for which Fe, Co and Cu are of particular biological importance. We therefore took octahedral Co(III) as being typical of these, and of course octahedral coordination is by far the most common geometry for those ionophores whose structures are known.

If ulapualide A is indeed an ionophore, then we can speculate that the natural stereoisomer will be the one which is best able to form a metal chelate, i.e. the stereoisomer which shows the lowest strain energy for a ulapualide–metal complex. We have therefore used molecular mechanics calculations with Co(III) to try and determine which diastereomer of ualpualide A can form the lowest energy metal chelate. We would then predict that this will be the stereochemistry of naturally occurring ulapualide A.

Before progressing to examine all possible metal ion-ulapualide A complexes we focused our initial work on the use of a 'dummy' metal atom for complexation. This showed, for example, that only two of the three oxazole nitrogen centres in ulapualide A could complex to a metal atom at

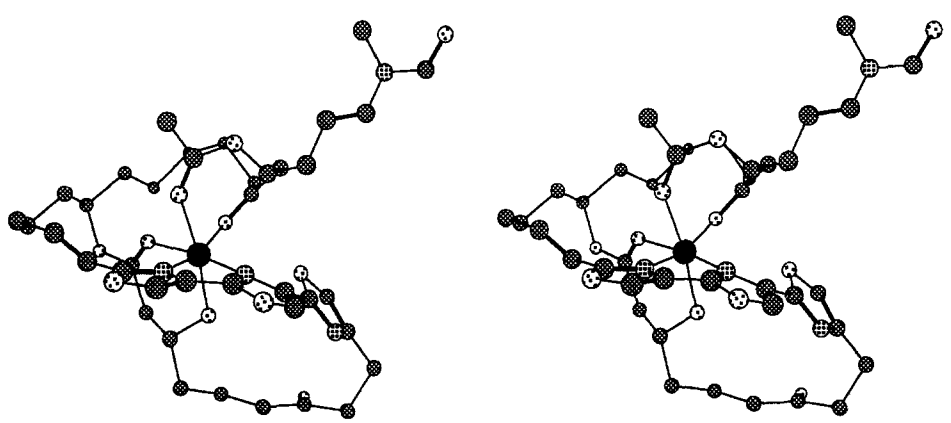


Fig. 3. A stereoview of the generic metal–ulapualide A complex (**6**) [the donor atoms used are C=O (1), OH (3), N (14), N (17), C=O (30) and OAc (32), to give the metal stereochemistry as *OC*-6-54-*A*].

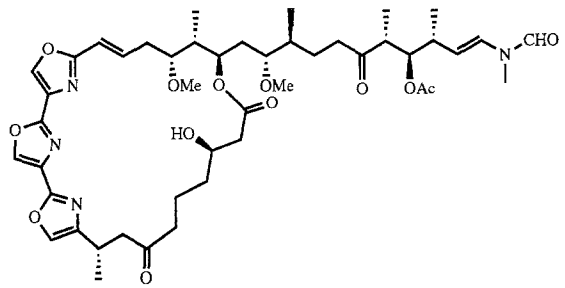


Fig. 4. Structure of ulapualide A with the stereochemistry as predicted in Table 2.

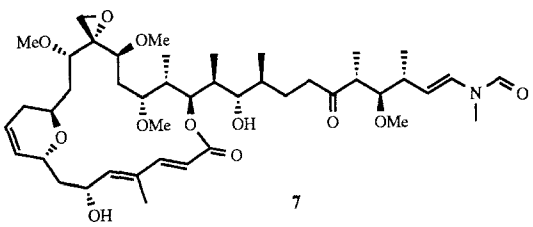
one time (Fig. 2). In order to test the viability of metal complex formation, generic structure **5**, which incorporates only those features common to both the ulapualides and halichondramides, was wrapped around the dummy metal atom to form a rather arbitrarily chosen octahedral complex using two oxazole aza-nitrogen centres and four carbonyl oxygen donors. A conformational search on the complex then produced structure **6**; this is illustrated in energy-minimised form in Fig. 3.

Having obtained complex **6** the various side chain substituents in ulapualide A were added to the complex in sequence, one at a time. As each substituent was added both of the epimers were then considered and energy minimised; and in each case the epimer of higher energy was discarded. When all the substituents had been added, each stereocentre was inverted in turn and the structure re-minimised as a double check; some pairs of centres were also inverted where inspection showed that this was likely to produce a favourable structure. We were reassured to find that this double check did not alter any of the original stereochemical assignments. The resulting stereoisomer of ulapualide A obtained by this method is that shown in Fig. 4, with the 'energetic penalty' upon stereocentre inversions given in Table 2. Deletion of the dummy metal from the ulapualide complex and re-minimisation produced a local minimum of similar conformation to

TABLE 2
THE ENERGETIC PENALTY UPON STEREOCENTRE INVERSION FOR COMPLEX 6

Stereocentre	Predicted configuration	ΔE upon inversion (kJ mol^{-1})
C9-Me	S	4.2
C22-OMe	R	16.2
C23-Me	S	28.6
C26-OMe	R	14.9
C27-Me	S	3.0
C31-Me	R	2.3
C32-OAc	R	>500
C33-Me	R	15.2
C22 and C33	–	53.1
C31 and C33	–	10.9

The stereochemistry given is relative to an arbitrarily defined *R* stereocentre at the C24 ring junction.



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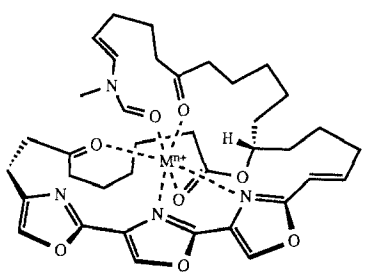
that of the complex and only 12 kJ mol^{-1} higher in energy than the global minimum energy for the ligand alone. This would seem to indicate that the generic structure **5** is at least capable of metal complex formation.

At the time that our work was in progress we became aware of the related marine metabolite, scytophycin B (**7**) [19], whose structure showed a similar side chain, terminating in a formyl enamine moiety, to that found in ulapualide A. In addition, eight of the stereocentres in the side chain of scytophycin overlapped with the corresponding portion of ulapualide A (see structures).



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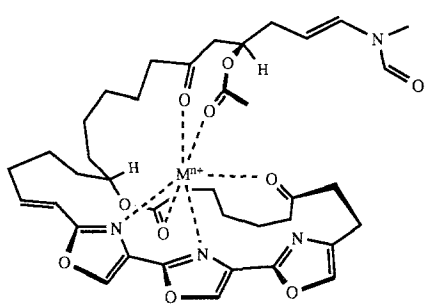

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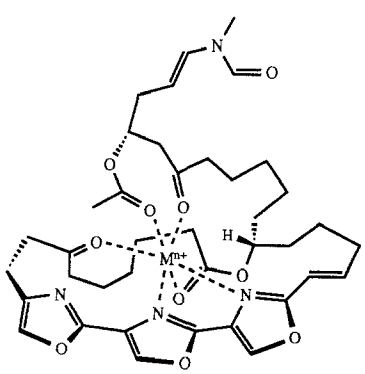

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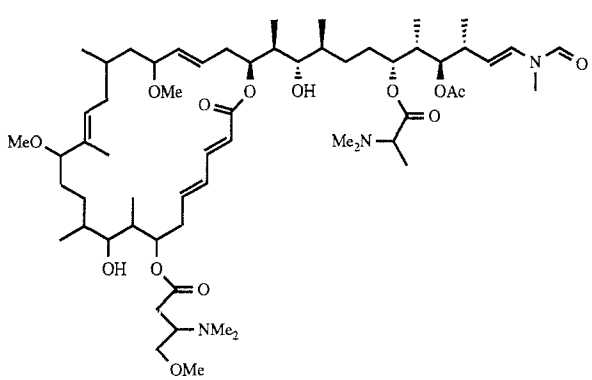

11 Aplyronine

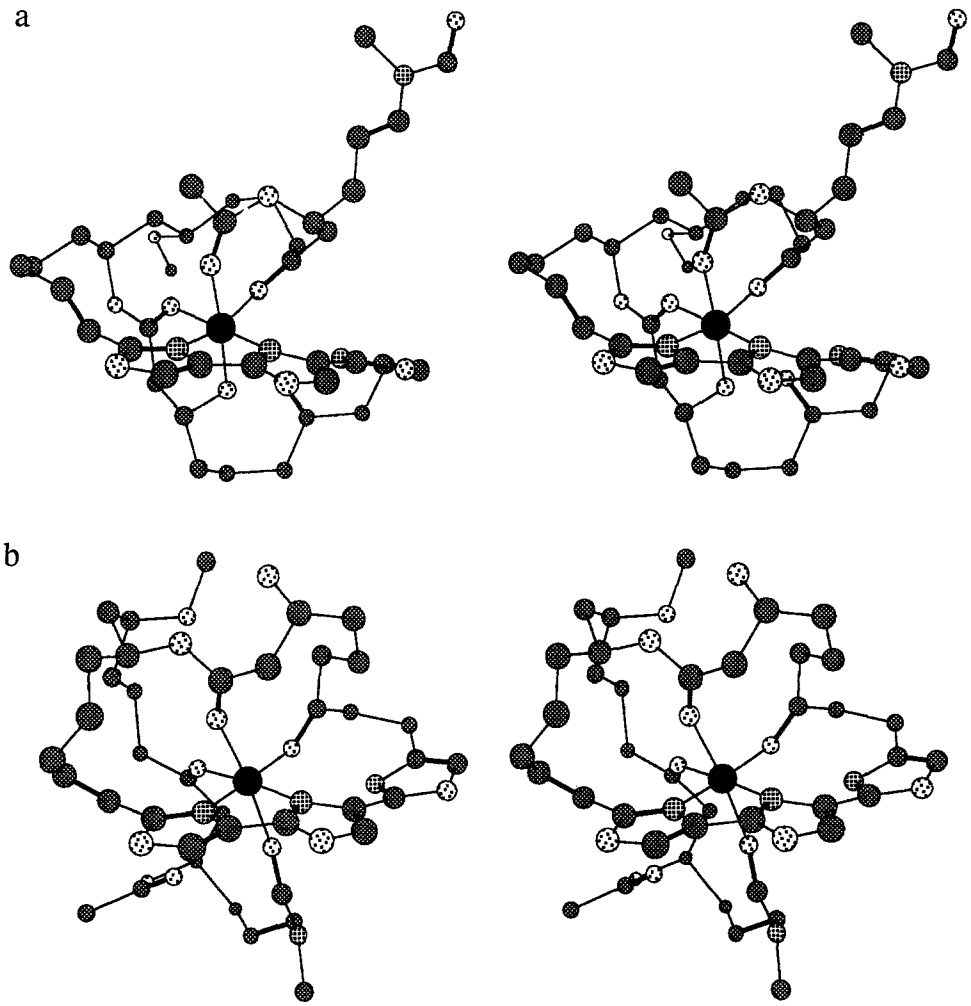

Fig. 5. Stereoviews of the generic complexes **6**, **8**, **9** and **10**. (a) Complex **6**; the donor atoms and metal stereochemistry are as for Fig. 3. (b) Complex **8**; the donor atoms used are C=O (1), C=O (7), N (14), N (17), C=O (30) and C=O (37), to give the metal stereochemistry as *OC*-6-25-*C*.

The comparison was all the more startling because the relative stereochemistry of scytophycin (unambigiously obtained by an X-ray crystal structure determination of a transformation product) was identical to that predicted for ulapualide A by our crude chelation model!

Clearly at this stage in our study we were greatly encouraged, and we decided to investigate the metal ion–ulapualide A complex formation more fully. Thus, using the generic structure **5** and the full Co(III) model given in Table 1, twenty-four complexes, using a variety of donor atom and metal centre geometries, were examined systematically [20]. Of these, only four (structures **6**, **8**, **9**, and **10**)) possessed reasonable global minimum energies, and the energy-minimised structures for these are shown in Figs. 5a–d (the donor atoms and metal centre stereochemistry for each complex is given in the figures). A considerable amount of time was given to the location of viable

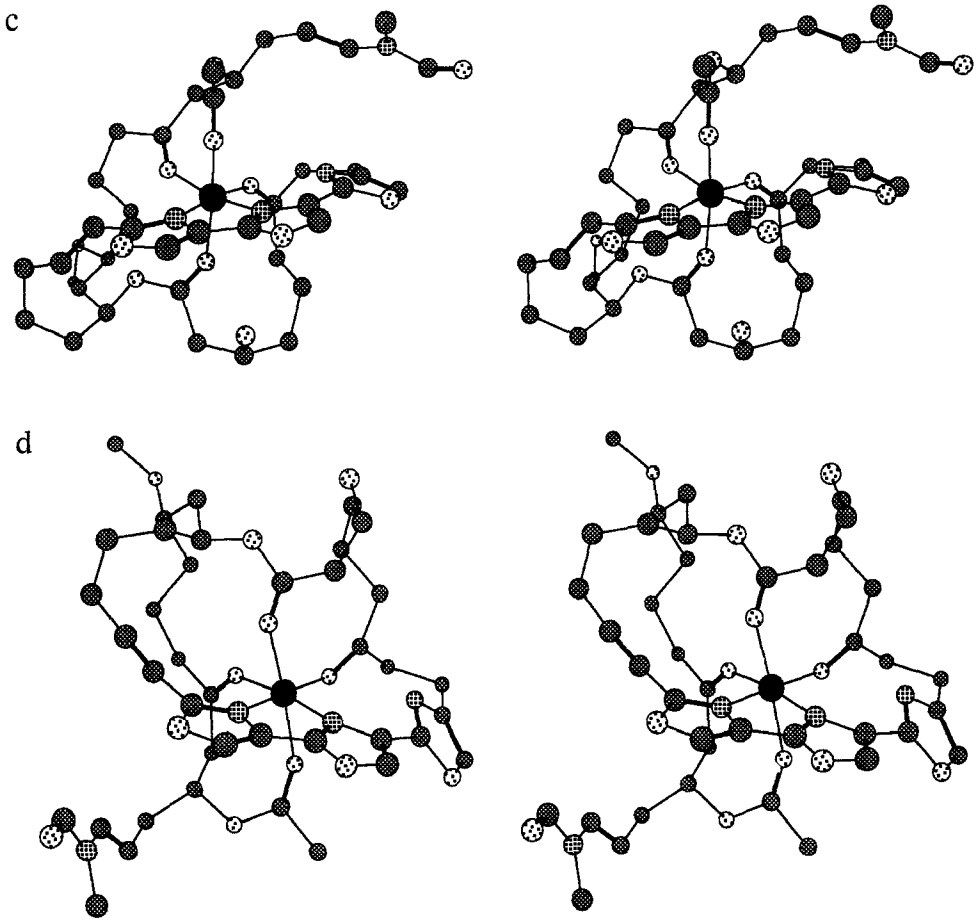

Fig. 5. (continued). (c) Complex **9**; the donor atoms used are C=O (1), C=O (7), N (14), N (17), C=O (30) and OAc (32) to give the metal stereochemistry as *OC*-6-25-*A*. (d) Complex **10**; the donor atoms used are as for (c), but the metal stereochemistry is *OC*-6-25-*C*.

complexes, and model building and dynamics simulations [21] of the complexation process (see Fig. 6) failed to locate any other complex geometries. Generally it was found that the use of N14 and N17 in the ulapualide was preferred over N11 and N14, while oxy-carbonyl donors were found to be preferable to ether (methoxyl) donors due to their lower steric requirements.

Having identified the four complex geometries, i.e. **6**, **8**, **9** and **10**, these were then examined separately for their ability to tolerate metals of different sizes [22]. Simultaneous linear variation of metal ligand bond length and metal atom size (Van der Waals radii) for each of the complexes produced the internal energy versus metal ligand bond length curves shown in Fig. 7. It can be seen from these curves that for each of the complexes the ideal metal ligand bond length lies in the region of 2.0–2.3 Å, corresponding to the strain-free bond lengths for many typical M^{2+} and M^{3+} ions, e.g. Co(II), 2.0 Å; Fe(III), 2.0 Å; Cr(III), 2.08 Å; Ni(II), 2.1 Å; Zn(II), 2.1 Å; Cd(II), 2.25 Å; and Ca(II), 2.28 Å [23–26].

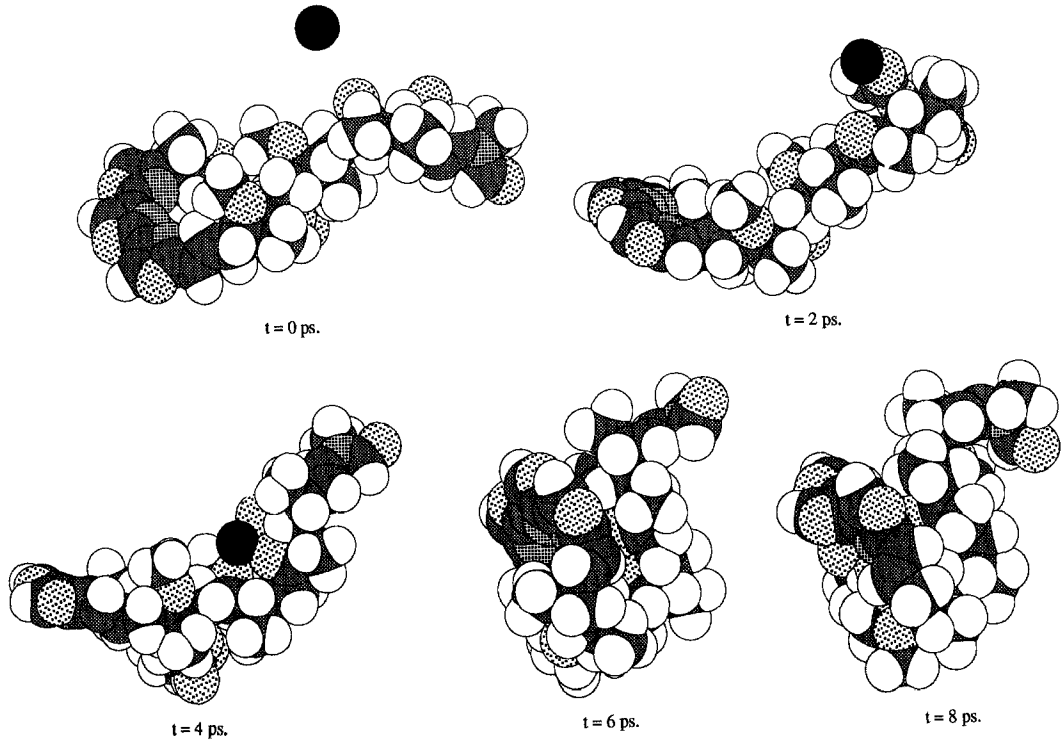

Fig. 6. A representative molecular dynamics simulation; the structures shown are taken at 2 ps intervals starting from t = 0.

The various ulapualide A side chain substituents were then added to each of the complexes **6**, **8**, **9** and **10**, and the optimum stereochemical arrangements for metal complexation were determined in a manner analogous to that already described above. The result of this exercise was that only structures **8** and **9** were able to tolerate the introduction of the full complement of substitu-

TABLE 3
THE ENERGETIC PENALTY UPON STEREOCENTRE INVERSION FOR COMPLEXES **8** AND **9**

Stereocentre	Predicted configuration for **8**	ΔE upon inversion for **8** (kJ mol^{-1})	Predicted configuration for **9**	ΔE upon inversion for **9** (kJ mol^{-1})
3-OH	R	5.4	R	30.5
C9-Me	S	6.4	R	8.1
C22-OMe	R	1.2	R	4.4
C23-Me	S	10.9	S	8.2
C26-OMe	R	4.8	S	17.9
C27-Me	S	65.1	S	36.7
C31-Me	R	28.1	R	3.3
C32-OAc	R	14.4	R	36.9
C33-Me	R	6.3	S	1.3

The stereochemistry given is relative to an arbitrarily defined *R* stereocentre at the C24 ring junction.

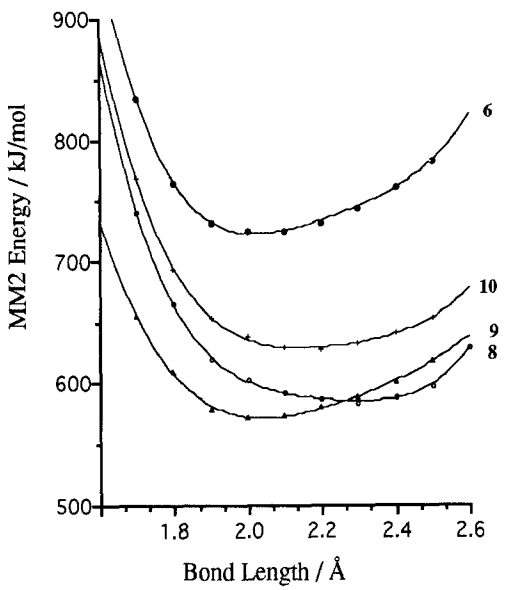

Fig. 7. A plot of internal energy of the complex versus metal-ligand bond length for the generic complexes **6**, **8**, **9** and **10**.

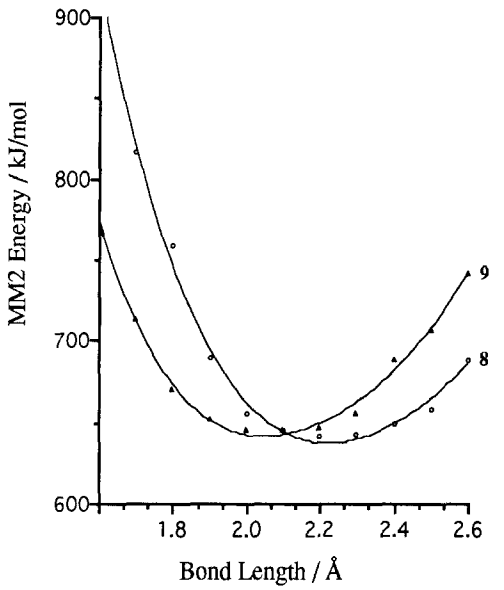

Fig. 8. A plot of internal energy of the complex versus metal-ligand bond length for the fully substituted complexes and **9**.

ents found in ulapualide A, without a substantial increase in complex energy. Complexes **8** and **9** were found to be of equivalent energy, while complexes **6** and **10** were found to be 148 and 50 kJ mol^{-1}, respectively, higher in energy than complex **9**. The predicted relative stereochemistries for metal–ulapualide A complexes **8** and **9** and the energetic penalty upon stereocentre inversion are given in Table 3. It can be seen that the two differing complexes predict the same relative stereochemistry for seven of the ten chiral centres in the molecule. In addition complex **8** agrees not only with our original simple model above, but also with the stereochemistry of scytophycin B (**5**). Complex **8** has one other advantage, in that it does not require the use of the acetate carbonyl oxy centre to complex to the metal. This structural feature is not present in the halichondramide series of compounds, which exhibit the same biological properties and potency as ulapualide A.

Finally we repeated the metal size variation and re-minimisation with the complexes **8** and **9**, but incorporating their side chain substituents with stereochemistries as predicted above. The resulting curves are shown in Fig. 8, and it can be seen that these are broadly similar to those obtained before. It is rather interesting to note that the curves cross at 2.15 Å leading one to predict differing stereochemistries for ulapualide A (i.e. either **8** or **9**) depending upon which metal ions the ulapualides interact with in vivo. We believe that the larger cavity of **8** cf. **9** is in its favour in this respect, since calcium chelating macrolides are well known for their cytotoxic behaviour in vivo as are the ulapualides. The final energy-minimised structures for **8** and **9** are given in Fig. 9.

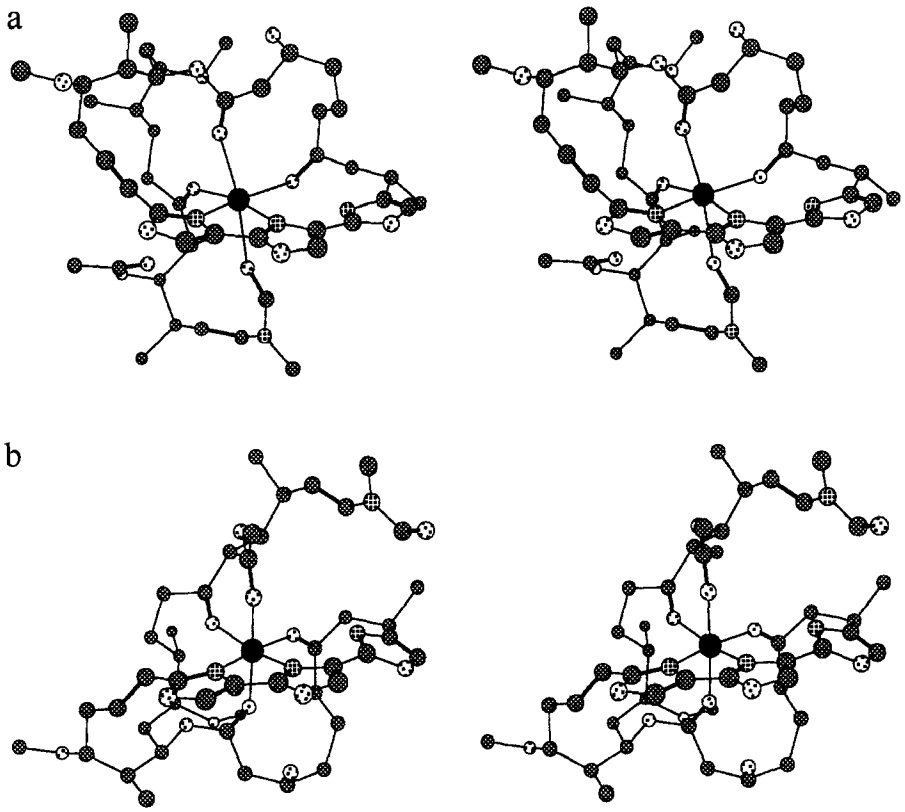

Fig. 9. Final stereoviews of: (a) complex **8**, and (b) complex **9**, with all substituents in place and stereochemistry as predicted.

CONCLUSIONS

Whilst Nature will clearly not always construct the lowest energy system, the importance that the stereochemistry of substituents plays in predisposing the ligands in natural products towards complexation has been recognised previously [27,28]. In at least one other case [29], the stereochemistry of a natural product has been found to be that which produces the lowest energy chelate. In view of this precedent we have used molecular mechanics calculations on metal ion–ulapualide A complexes in a predictive sense to determine the likely stereochemistry of ulapualide A (**1**). In view of the consistency of the results and the similarity in stereochemistry to related marine natural products we have predicted for ulapualide A, e.g. scytophycin B (**5**), we propose that the stereochemistry of ulapualide A is as shown in Fig. 4. The recent disclosure of the existence of an additional marine natural product, viz. aplyronine (**11**), which also shows remarkable similarity to our predicted stereochemistry for ulapualide A, adds further evidence for our stereochemical model. Here the stereocentres defined in structure **11** were determined by a combination of degradation and partial synthesis [30]. We have used these molecular mechanics studies in conjunction with our own preliminary synthetic work in this area which would seem to be in agreement with our stereochemical assignment for ulapualide A [9a, b,31].

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REFERENCES

1 Roesener, J.A. and Scheuer, P.J., J. Am. Chem. Soc., 108 (1986) 846.
2 Matsunaga, S., Fusetani, N., Hashimoto, K., Koseki, K. and Noma, M., J. Am. Chem. Soc., 108 (1986) 847.
3 Matsunaga, S., Fusetani, N., Hashimoto, K., Koseki, K., Noma, M., Noguchi, H. and Sankawa, U., J. Org. Chem., 54 (1989) 1360.
4 Kernan, M.R. and Faulkner, D.J., Tetrahedron Lett., 28 (1987) 2809.
5 Kernan, M.R., Molinski, T.F. and Faulkner, D.J., J. Org. Chem., 53 (1988), 5014.
6 Fusetani, N., Yasumuro, K., Matsunaga, S. and Hashimoto, K., Tetrahedron Lett., 30 (1989) 2809.
7 Fusetani, N., Sugawara, T., Matsunaga, S. and Hirota, H., J. Org. Chem., 56 (1991) 4971.
8 For a review see: Michael, J.P. and Pattenden, G., Marine metabolites and metal ion chelation: The facts and the fantasies, Angew. Chem. Int. Ed. Engl., 105 (1993) 1.
9 a. Knight, D.W., Pattenden, G. and Rippon, D.E., Synlett, 1 (1990) 36.
b. Keifel, M.J., Maddock, J. and Pattenden, G., Tetrahedron Lett., 33 (1992) 3227.
10 Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, M., Caufield, C., Chang, G., Hendrickson, T. and Still, W.C., J. Comp. Chem., 11 (1990) 440.
11 Allinger, N.L., J. Am. Chem. Soc., 99 (1977) 8127.
12 DeHayes, L.J. and Busch, D.H., Inorg. Chem., 12 (1973) 1505.
13 Laier, T. and Larsen, E., Acta Chem. Scand., 33 (1979) 257.
14 Lin, W.-K., Alcock, N.W. and Busch, D.H., J. Am. Chem. Soc., 113 (1991) 7603.
15 Kistenmacher, T.J., Marzilli, L.G. and Marzilli, P.A., Inorg. Chem., 13 (1974) 2089.
16 Winkelman, G., van der Helm, D. and Neilands, J.B., Iron Transport in Microbes, Plants and Animals, VCH, New York, 1987, p. 416.
17 Initially the calculations using a 'dummy' metal centre were performed using the COSMIC force field. These results were then repeated using the MM2 force field and it is these data that are presented here.
18 Polak, E. and Ribière, G., Rev. Franç. Inform. Rech. Opérat., 16 (1969) 35.
19 Ishibashi, M., Moore, R.E., Patterson, G.M.L., Xu, C. and Clardy, J., J. Org. Chem., 51 (1986) 5300.
20 In principle for a given set of six differing ligands there are 30 different stereoisomers possible. However, in this case the topological constraints of the macrocyclic ligand meant that there were rarely more than two complexes that could be formed for a given set of donor atoms. Where the formation of a complex would later prevent the inversion of a stereocentre due to topological constraints, the alternative configuration was considered at this stage.
21 Dynamics simulations were carried out using the standard MM2 force field as implemented within MACROMODEL. The metal was taken as a hard sphere (radius 1.8 Å, charge +2, $\varepsilon = 0.228$) to crudely approximate to Ca^{2+}. Simulations utilised a 0.1 fs time step and ran over 30 ps at 300 K, non-bonded cut-offs were extended to 20 Å for van der Waals and 20 Å for electrostatic interactions to maintain stability during the simulation. All of these dynamics simulations were initiated with the uncomplexed metal. The Ca(II) model was chosen in order to avoid defining metal-ligands bonds in the hope that new low-energy complexes, i.e. other than **6**, **8**, **9**, and **10**, would be located. In the event, no new complexes were located, and most were found to be similar to either **7** or **8**. Once formed, these complexes were stable to further dynamics simulations, and donor atom exchange or metal atom extrusion was not observed.
22 This idea is by no means new; see for example Hancock, R.D., Acc. Chem. Res., 23 (1990) 253 and references therein.
23 Thöm, V.J., Fox, C.C., Boeyens, J.C.A. and Hancock, R.D., J. Am. Chem. Soc., 106 (1984) 5947.
24 Hancock, R.D., Pure Appl. Chem., 58 (1986) 1445.
25 Hancock, R.D., Weaving, J.S. and Marques, H.M., J. Chem. Soc., Chem.Commun., (1989) 1176.
26 Vedani, A. and Huhta, D.W., J. Am. Chem. Soc., 112 (1990) 4759.
27 Smith, P.W. and Still, W.C., J. Am. Chem. Soc., 110 (1988) 7917.
28 Erickson, S.D. and Still, W.C., Tetrahedron Lett., 31 (1990) 4253.

29 Still, W.C., Hauck, P. and Kempf, D., Tetrahedron Lett., 28 (1987) 2817.
30 Yamada, K., Ojika, M., Kigoshi, H., Yoshida, Y., Nisiwaki, M.,Tsukada, I., Ishigaki, T. and Ogawa, T., Proceedings of the 7th International Symposium on Marine Natural Products, July 1992, Capri, Italy.
31 Attempts by us to crystallise *bis*- and *tris*-oxazole derivatives have not yielded samples suitable for X-ray structure determinations. We have not therefore been able to test the suitability of the MM2 force field for modelling these systems.