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Conformational studies of A23187 with mono-, di- and trivalent metal ions by circular dichroism spectroscopy

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CD; Ionophore-metal ion complex; Conformational analysis; Coordination structure

CD studies carried out on A23187 indicate a solvent-dependent conformation for the free acid. Alkali metal ions were found to bind to the ionophore weakly. Divalent metal ions such as Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and Co^{2+} and trivalent lanthanide metal ions like La^{3+} were found to form predominantly 2:1 (ionophore-metal ion) complexes at low concentrations of metal ions, but both 2:1 and 1:1 complexes were formed with increasing salt concentration. Mg^{2+} and Co^{2+} exhibit similar CD behaviour that differs from that observed for the other divalent and lanthanide metal ions. The structure of 2:1 complexes involves two ligand molecules coordinated to the metal ion through the carboxylate oxygen, benzoxazole nitrogen and keto-pyrrole oxygen from each ligand molecule along with one or more solvent molecules. Values of the binding constant were determined for 2:1 complexes of the ionophore with divalent and lanthanide metal ions.

1. Introduction

A23187 (also called calcimycin) isolated from the bacterium *Streptomyces chartreusensis* is a carboxylic carrier. It has been shown to have a pronounced preference for divalent over monovalent cations [1]. It is a strong uncoupler of oxidative phosphorylation and inhibitor of ATPase in rat liver mitochondria [2]. Besides this, it was shown to induce secretion of enzymes, hormones, biogenic amines and neurotransmitters, activate eggs, cause proliferation of lymphocytes, induce synchrony of growth, affect membrane fusion and cell morphology and interfere with cellular metabolism at many different levels [3].

The structure consists of a keto-pyrrole portion, a spiroketal portion and a benzoxazole portion (substituted with a methylamino group and a carboxylic acid group). X-ray studies of the free acid by Chaney et al. [4] showed the presence of three intramolecular hydrogen bonds, one between the pyrrole NH and carboxylate oxygen, the second between the carboxylate proton and benzoxazole nitrogen and the third between the carboxylate oxygen and the proton from the secondary amino nitrogen. Of the two reported crystal structures for 2:1 Ca^{2+} complexes of A23187 [5,6], that of Smith and Duax [6] appears to be the correct one [7]. The dihydrate contains four oxygen atoms from the two ligand molecules (one carboxylate oxygen and a carbonyl oxygen from each ligand molecule) arranged nearly in a square, with two nitrogens (benzoxazole ring nitrogen from each ligand molecule) in the perpendicular plane bisecting the carboxylate oxygen and the carbonyl oxygen. The coordination number of Ca^{2+} in this case is seven, water taking the apex at the other end.

Pfeiffer et al. [1] have carried out some preliminary studies on this ionophore in the presence of divalent metal ions using circular dichroism

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(CD) spectroscopy. The present work deals with the structure of complexes of A23187 with monovalent (Li^+, Na^+ and K^+), divalent (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and Co^{2+}) and trivalent (La^{3+}, Eu^{3+} and Yb^{3+}) cations studied using CD spectroscopy.

2. Materials and methods

A23187 was obtained as the free acid from Sigma and used as such. All monovalent and divalent metal ions were used as perchlorates obtained from Alfa Laboratories (U.S.A.). Whereas the lithium, sodium, potassium and cobalt perchlorates were anhydrous compounds, those of divalent metal ions were obtained as hexahydrates. Lanthanide oxides purchased from Alfa Laboratories were used as nitrates with six molecules of water of crystallisation. The perchlorates were dried over P_2O_5 for several hours before use.

CD spectra were recorded on a Jasco J-20 automatic recording spectropolarimeter operating at 20 °C. A typical experiment made use of about 100–200 μM ionophore. Analar grade solvents such as methanol from Merck (India), chloroform from Glaxo Laboratories (India) and *n*-hexane from SD's were used for some of the CD measurements. Analar grade acetonitrile from Fluka was used for the CD studies. Molar ellipticity values were calculated using the relation

$$[\theta]_\lambda = \frac{100\,\theta_{\text{deg.}}}{Cl}$$

where $[\theta]_\lambda$ is the molar ellipticity value determined at a particular wavelength expressed in degree cm² dmol⁻¹, C the concentration in mol/l, l the path length in dm and $\theta_{\text{deg.}}$ the rotation in degrees observed at the specific wavelength. The values obtained for each metal ion were from at least two to three experiments involving different concentrations of the ionophore in the concentration range mentioned above.

3. Results

3.1. CD studies

The CD spectra of the ionophore in *n*-hexane, chloroform, acetonitrile and methanol are given in fig. 1. In all these cases three bands are observed at 275, 290 and 375 nm, respectively. The ultraviolet spectrum of the ionophore in acetonitrile exhibits a band at 377 nm corresponding to the π-π^* transition of the *N*-methylanthranilic acid part of the benzoxazole portion (molar extinction coefficient 9170) and another one with two shoulders centred at 278 nm (molar extinction coefficient 23200) corresponding to the n-π^* transition of the benzoxazole ring and keto-pyrrole group. The observed CD band at 375 nm corresponds to the *N*-methylanthranilic acid part of the benzoxazole portion and that at 275 nm to the benzoxazole ring portion involving nitrogen. The relative signs and magnitudes of these bands in the four solvents used above are listed in table 1. On going from a non-polar solvent such as *n*-hexane to a polar solvent like methanol, the large negative band at 276 nm for *n*-hexane changes to a large positive one at 290 nm. Chloroform and acetonitrile exhibit negative bands (at 290 nm) but the magnitudes are less than that in *n*-hexane in the order *n*-hexane > chloroform > acetonitrile.

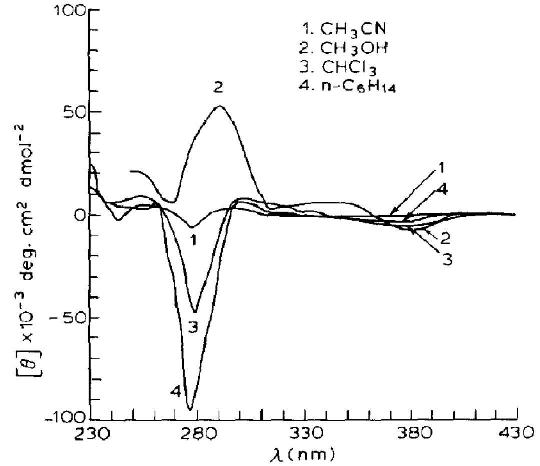

Fig. 1. CD spectra of A23187 in different solvents.

Addition of monovalent cations like Li^+, Na^+ and K^+ to A23187 in acetonitrile was studied. Monovalent cations can bind effectively to the carboxylate oxygen and carbonyl oxygen but not to the benzoxazole nitrogen. Accordingly, the CD spectra of the ionophore in the presence of mono-

Table 1

Molar ellipticity values of the free acid in different solvents and its complexes with mono-, di- and trivalent metal ions in acetonitrile

Each $[\theta]$ value is accompanied by the sign and wavelength of the CD band. For the complexes, it is the $\Delta[\theta]_{max}$ values ($[\theta]_{sample} - [\theta]_{free\,acid}$) that are given here.

System	Molar ellipticity (degree cm^2 dmol^{-1})			
A23187 in methanol	(−)	(+)		(−)
	380	291		270
	−7080	52762		5478
A23187 in acetonitrile	(−)	(+)		(−)
	370	295		278
	−834	2522		−6366
A23187 in chloroform	(−)	(+)		(−)
	375	301		280
	−5009	8682		47085
A23187 in *n*-hexane	(−)	(+)		(−)
	370	301		277
	−2963	6915		−95332
A23187-Li$^+$	(−)	(+)		(−)
	383	293		275
	470	−1750		−6800
A23187-Na$^+$	(−)	(+)		(−)
	372	299		280
	300	−180		−2100
A23187-K$^+$	(−)	(+)		(−)
	375	294		278
	−294	3571		2981
A23187-Mg^{2+}	(+)	(+)		(−)
	365	296		255
	2297	134600		16400
A23187-Ca^{2+}	(+)	(+)		(+)
	370	304		272
	22000	40500		45200
A23187-Sr^{2+}	(+)	(+)		(−)
	368	300		277
	12400	19200		12600
A23187-Ba^{2+}	(+)	(+)		(−)
	360	304		282
	2850	7600		−1750
A23187-Co^{2+}	(+)	(+)		(+)
	350	300		255
	3200	113000		15600
A23187-La^{3+}	(+)	(−)		(+)
	365	318		270
	31000	−102000		101000
A23187-Eu^{3+}	(+)	(−)	(+)	(+)
	366	322	302	270
	16500	−24500	42500	56500
A23187-Yb^{3+}	(+)	(−)	(+)	
	345	324	303	
	5000	−43500	91000	

valent cations can be expected to show some changes near the 375 and 290 nm region. These bands exhibit changes that are inconsistent with the increase in metal ion concentration (table 1). They do not change in a regular fashion. The observed changes are too small in magnitude, within the range of experimental error, to be resolved unambiguously.

Divalent metal ions such as Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and Co^{2+} were added to A23187 in increasing amounts and their effects were studied by CD in acetonitrile as the solvent. The results are summarized in table 1 and fig. 2. Invariably all these divalent metal ions give rise to changes in the CD bands around 360, 300 and 275 nm. Of these Ca^{2+} shows remarkable changes in all three regions. A broad band at 370 nm and another at 304 nm are the essential features of the calcium complex CD spectra. The CD curve also indicates an asymmetric structure for the complex. The CD spectra of the Mg^{2+} complex exhibit a small change in the 365 nm region and a large change in the 296 nm band. The nature of the CD curve indicates an ordered structure. The cobalt complex exhibits similar CD spectra. The observed effects for the Sr^{2+} and Ba^{2+} complexes of this

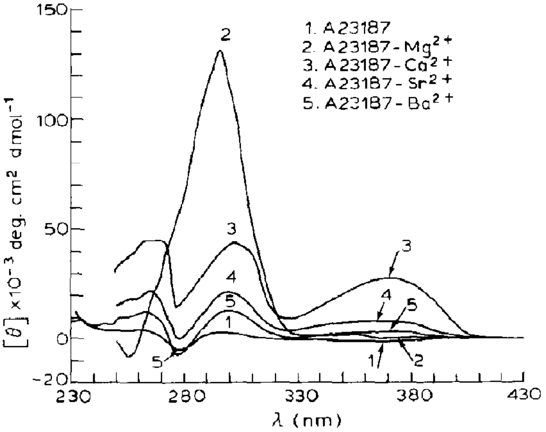

Fig. 2. CD spectra of A23187 in the presence of alkaline earth metal ions in acetonitrile. Concentrations of the ionophore used varied from 80 μM–130 μM. Plots obtained were for concentrations of metal ions corresponding to maximum $[\theta]$ ($[\theta]_{max}$) values, which were normally of the ratio 1:1 (ionophore : metal ion).

ionophore in the CD spectra resemble that of the calcium complex (but not those of Mg^{2+} and Co^{2+}), however, with lesser magnitude in all three regions concerned.

Addition of lanthanides to A23187 in acetonitrile gave rise to complex CD spectra. The ultraviolet spectral bands observed for these complexes are given in table 2. The unassigned peak at 245 nm region in the ultraviolet spectra of the three lanthanide complexes probably arises from the aromatic portion of the ionophore molecule. Although the ionic radii [8] of these lanthanide metal ions (La^{3+}, 1.016 Å; Eu^{3+}, 0.95 Å; Yb^{3+}, 0.86 Å) are in the range of those for divalent metal ions, the CD curves obtained for La^{3+}, Eu^{3+} and Yb^{3+} complexes of this ionophore are different from what was observed for the divalent metal ions (fig. 3 and table 1). For all the lanthanide metal ions studied, the band at 360 nm is positive, the magnitude of change in the molar ellipticity values of which decreases in the order $La^{3+} > Eu^{3+} > Yb^{3+}$. A special feature is the negative band around 320 nm observed for all three metal ions, the order of magnitude of this negative band being $La^{3+} > Eu^{3+} > Yb^{3+}$. Since the ultraviolet spectra of the lanthanides show a peak at 309 nm (except for ytterbium which exhibits a peak at 316 nm), this should be the keto-pyrrole band of the lanthanide complexes, observed around 290–300 nm in the case of divalent metal ion complexes. Also, La^{3+} and Eu^{3+} complexes exhibit a large positive band at 270 nm whereas the Yb^{3+} complex gives rise to a positive band at 303 nm.

3.2. *Stoichiometry of the complexes and binding constants*

Plots of $\Delta[\theta]$ ($[\theta]_{\text{sample}} - [\theta]_{\text{free acid}}$, where $[\theta]_{\text{sample}}$ is the value obtained for the ionophore at a given concentration of the metal ion) vs. the ratio of the metal ion added to the total concentration of the ionophore (ρ) were obtained for all the metal ions studied. $\Delta[\theta]$ values were obtained for one or more of the observed CD bands for each metal ion. However, only the titration curves obtained for the CD band corresponding to the keto group was considered for the calculations. Usually a hyperbolic curve is obtained which becomes asymptotic after a certain value of ρ. In all the cases studied this value was observed to be 0.5. Hence, formation of 2 : 1 (ionophore/metal ion) complexes was inferred at low concentrations of metal ions. Divalent cations and La^{3+} and Eu^{3+} indicate the formation of 2 : 1 complexes. Monovalent cations and Yb^{3+} give irregular CD titration plots. The CD titration curves obtained in these cases were not hyperbolic. Even at low concentrations of metal ions, wavy curves were obtained. A typical titration plot observed is given for the A23187-Mg^{2+} complex at $[\theta]_{296}$ (fig. 4). Certain metal ions such as Ba^{2+}, Co^{2+}, Eu^{3+} and Yb^{3+} show deviations from hyperbolic nature in the CD titration curve (for the keto band) after a ρ value > 0.5, implying probably the formation of more of the 1 : 1 complexes as the concentration of the metal ion is increased.

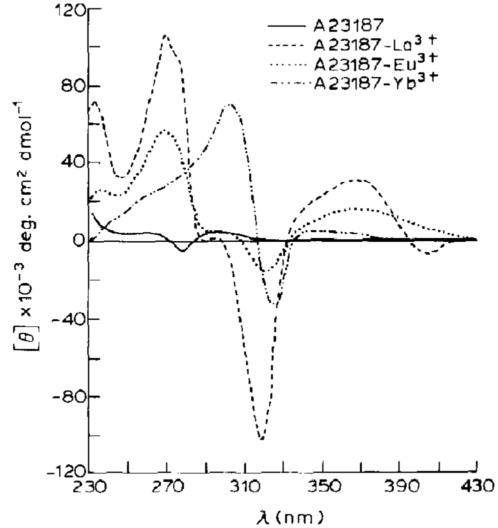

Fig. 3. CD spectra of A23187 with La^{3+}, Eu^{3+} and Yb^{3+} in acetonitrile. Plots were obtained as mentioned in fig. 2.

Table 2

Ultraviolet spectral bands (in nm) of A23187-Ln^{3+} complexes

System	*N*-Methylanthranilic acid part of benzoxazole	Keto-pyrrole	Benzoxazole	Unassigned
A23187-La^{3+}	382	309	280	245
A23187-Eu^{3+}	386	309	271	243
A23187-Yb^{3+}	384	316	272	232

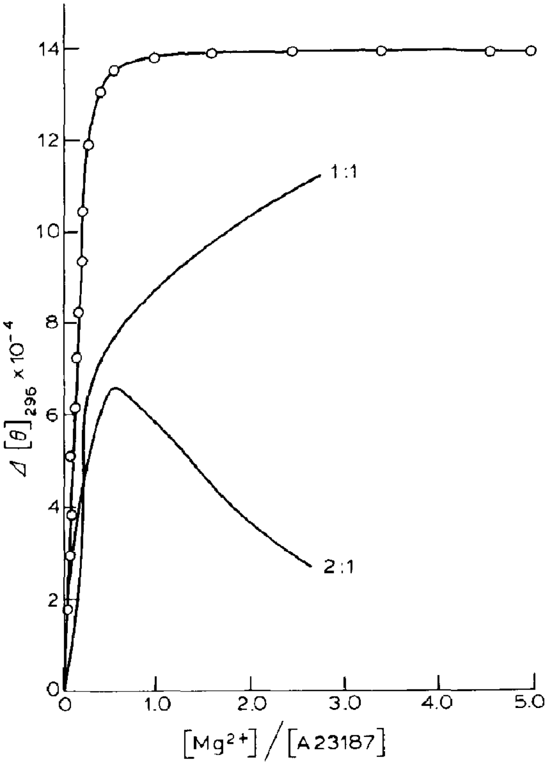

Fig. 4. CD titration plot of A23187-Mg^{2+} for the $\Delta[\theta]$ values obtained at 296 nm. The plot with the points is the experimental curve obtained. Those without the points are the computer-generated plots obtained from a four-parameter fit for the 1:1 and 2:1 complexes. The best fit was obtained for $K_2 < 4K_1$ and $\Delta[\theta]_1 = \Delta[\theta]_2$. $K_2 = 1.67 \times 10^{-6}$ M; $K_1 = 1.11 \times 10^{-4}$ M; $\Delta[\theta]_1 \simeq \Delta[\theta]_2 = 1.303 \times 10^5$.

Scatchard plots were constructed based upon the method of Reuban [9]. Data from CD titration curves for ρ values less than 0.5 (corresponding to 2:1 complexes) were considered for constructing Scatchard plots with the explicit assumption that only the binding constant values for 2:1 complexes were calculated. When $(\Delta[\theta]/(\Delta[\theta]_{\max} - \Delta[\theta])) \cdot 1/[\mathrm{M}^{n+}]$ (corresponding to r/C_f, where r is the ratio of bound to total number of ligand molecules and C_f the concentration of free ligand molecules) was plotted vs. the quantity $(\Delta[\theta][\mathrm{A23187}])/(\Delta[\theta]_{\max}[\mathrm{M}^{n+}])$, corresponding to r, convex curves were obtained for all the divalent and lanthanide metal ions studied. A non-linearity in the Scatchard plots indicated the coexistence of species with different stoichiometries. If K_1 and K_2 are the dissociation constants for the 1:1 and 2:1 complexes, respectively, negative ($K_2 > 4K_1$) or positive ($K_2 < 4K_1$) cooperativity will cause a curvature in the line making it concave or convex, respectively. A detailed discussion of these non-linear Scatchard plots is given by Reuban [9]. Convex curves obtained for all the divalent metal ions and La^{3+} indicated positive cooperativity where the condition $K_2 < 4K_1$ was satisfied. A four-parameter (K_1, K_2, $\Delta[\theta]_1$ and $\Delta[\theta]_2$, where $\Delta[\theta]_1$ and $\Delta[\theta]_2$ are the limiting molar ellipticity values of the 1:1 and 2:1 complex, respectively) computer fit was attempted for all the above-mentioned metal ions. Initially, approximate values of K_1 and K_2 and a range for $\Delta[\theta]_1$ and $\Delta[\theta]_2$ should be defined. By means of an iterative procedure the titration curve of 'best fit' is obtained by considering the values for $\Delta[\theta]_1$ and $\Delta[\theta]_2$, suitably incremented, which when added together should correspond to the experimentally observed one for each value of ρ. Then, a Scatchard plot obtained from these values should give the K_1 and K_2 values around the supposed values assumed before. If the correspondence between the experimentally observed and computer-generated values was not achieved the values for all four parameters were redefined once again and the procedure was repeated till a best fit was obtained. For systems of this type a good fit with the observed

Table 3

Binding constant values for 2:1 complexes of A23187 with metal ions

Scatchard plots were constructed from the data used for obtaining the CD titration plots at the wavelengths given above. Only the initial values of the CD titration plots corresponding to an ionophore/metal ion ratio of 2:1 were used.

System	CD maxima (nm)	Binding constant values ($\times 10^{-4}$) (mol^{-1})
A23187-Mg^{2+}	296	60.00
A23187-Ca^{2+}	304	39.84
A23187-Sr^{2+}	300	6.67
A23187-Ba^{2+}	304	0.53
A23187-Co^{2+}	300	22.50
A23187-La^{3+}	318	5.43
A23187-Eu^{3+}	322	4.03

CD titration curve can be expected for $K_2 < 4K_1$ and $\Delta[\theta]_1 \simeq \Delta[\theta]_2$. However, no satisfactory fit could be obtained for most of the ionophore-metal ion systems studied. However, for A23187-Mg^{2+} (and Ca^{2+}), a reasonable fit was achieved as shown in fig. 4. Similar studies carried out on molecules like nonactin [10] and valinomycin [11] have made use of these treatments with excellent results. The apparent stability constant values obtained for the 2 : 1 complexes are given in table 3. Stability constant values for the 1 : 1 complexes of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Co^{2+} and La^{3+} complexes were found to be low, being of the order of 10^2.

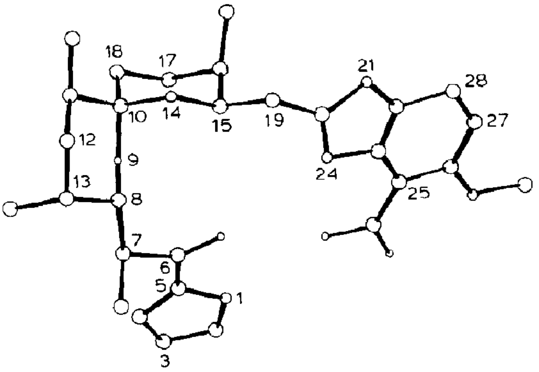

Fig. 5. Structure of A23187. The coordinates of Chaney et al. [7] were used.

4. Discussion

CD arises mainly because the right and left circularly polarized components of light exert a different net force on the electron on a helix, moving it up and down and sideways, hence enabling it to exhibit different absorption and refractive index for the above-mentioned polarized light. The electrons in a planar molecule are forced to move in the molecular plane so that the electric and magnetic dipole moments induced are perpendicular to each other, thus giving rise to no optical activity [12]. Although the relation between an asymmetric centre and optical activity is understood less well, a chromophore in the vicinity of an asymmetric centre gives rise to a CD band. A23187 possesses seven asymmetric centres of which C_7, C_8 and C_{15} (fig. 5) are in the vicinity of chromophores. Ligand atoms such as the carboxylate oxygen, keto oxygen and benzoxazole nitrogen are either in the vicinity or form a part of the chromophores. Hence, any interaction involving these chromophores results in rotation of the C_{15}–C_{19} and C_7–C_8 bonds which in turn give rise to pronounced effects on the CD bands arising from them. In the present study, such effects were observed in different solvents and in the presence of divalent and trivalent metal ions.

A solvent-dependent conformation for A23187 was observed through CD studies. Since the changes observed on going from *n*-hexane to methanol occur around the 275 and 290 nm regions, this should involve the two intramolecular hydrogen bonds, one between the pyrrole NH and carboxylate oxygen which brings together the two ends of the molecule resulting in a 'closed' structure (fig. 5) and the other between the benzoxazole nitrogen and carboxylate oxygen. Since the latter hydrogen bond does not contribute significantly to a drastic structural change when the hydrogen bonds are broken, the observed changes can be attributed only to the breaking of the hydrogen bond between the pyrrole NH and carboxylate oxygen. The intramolecular hydrogen bond that stabilises a closed structure in the non-polar solvent gives rise to an 'open' structure in a polar solvent. *n*-Hexane with a dielectric constant ($\epsilon_{20\,^\circ C}$) of 1.89 stabilises the intramolecular forces of attraction deployed through hydrogen bonds and hence favours a closed structure. Chloroform ($\epsilon - 4.81$ and donor number $\ll 10.0$) also exhibits the same behaviour. Acetonitrile ($\epsilon - 37.5$, donor number 14.1) and methanol ($\epsilon - 32.6$, donor number 33.8) should weaken these forces of attraction and therefore give rise to an open structure accompanied by rotation of the C_7–C_8 and C_{15}–C_{19} bonds aided by the rigid central spiroketal portion (fig. 5). While methanol exhibits large positive molar ellipticity values (at 290 and 270 nm), acetonitrile exhibits only small values indicating an intermediate structure for the ionophore in this solvent with the hydrogen bonds not being stabilised fully. Hence, the structure of the ionophore in a non-polar solvent like *n*-hexane may be similar to the X-ray structure for the free acid (fig. 5) determined by Chaney et al. [4].

Monovalent cations have been shown to coordinate to carbonyl oxygen forming stable complexes with ionophore like nonactin [7]. In A23187 the observed CD data for Li^+, Na^+ and K^+ were not clear enough to draw any quantitative conclusions. Association of monovalent cations with the carboxylate oxygen and carbonyl oxygen may result in the formation of either and/or 1 : 1 and 1 : 2 (ionophore/metal ion) complexes. In acetonitrile, this association may be too weak to induce the ionophore molecule to assume a favourable geometry for CD, the structure probably being the same as that of the free acid. Pfeiffer et al. [1] have also reported the stability constants for the monovalent cation complexes with the ionophore to follow the order $Li^+ > Na^+ > K^+ \simeq 0$.

The resemblance of the CD spectra of the ionophore in the presence of the divalent metal ions studied to that observed for the free acid in methanol indicates that considerable rotation occurs in the two hinge regions of the molecule, namely, C_7–C_8 and C_{15}–C_{19}, when two ligand molecules coordinate to the metal ions. Also, the intramolecular hydrogen bond between the pyrrole NH and carboxylate oxygen is broken in the free ligand molecule as it becomes complexed to a metal ion. From the changes observed in the CD bands corresponding to the benzoxazole and keto-pyrrole portions, it can be inferred that the two ligand molecules coordinate to the metal ion through the keto oxygen, carboxylate oxygen and benzoxazole nitrogen. Previously, X-ray [6] and NMR [3] have led to the proposed stabilisation of the complex structure involving two ligand molecules by two sets of intramolecular hydrogen bonds, one between the secondary amino nitrogen proton and carboxylate oxygen and the other between the carboxylate oxygen of one ligand molecule and the pyrrole NH proton of the other. A typical structure for the 2 : 1 (ionophore/metal ion) complex with Ca^{2+} is given in fig. 6. It can be seen that the single ionophore molecule itself appears curved, positioning the coordinating atoms in two planes perpendicular to each other (see section 1). In the absence of calcium, this may be the conformation for the open structure in methanol.

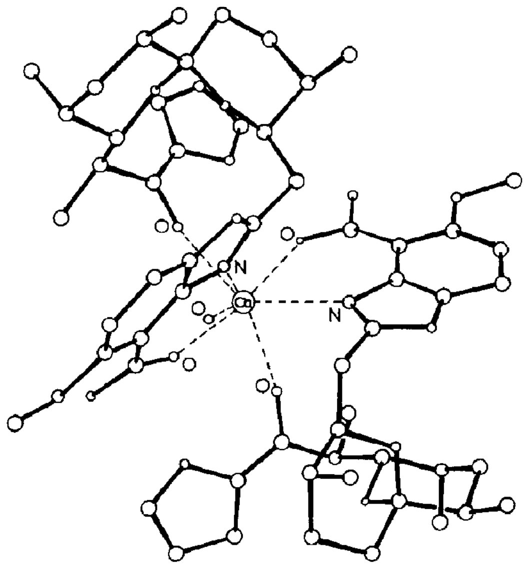

Fig. 6. Structure of A23187-Ca^{2+}. Coordinates for the structure of the dihydrate were those of Smith and Duax [7]. Dashed lines represent bonds from ligand atoms to Ca^{2+}.

Alkaline earth metal ions bind strongly to oxygen ligands from carboxylate and carbonyl groups. Only Mg^{2+} and Ca^{2+} form rather weak complexes with nitrogen ligands [13]. Among the divalent metal ions studied Co^{2+} is an exception, since it can bind to nitrogen and oxygen ligands quite efficiently. The similarity in CD spectra between Mg^{2+} and Co^{2+} complexes on the one hand and Ca^{2+}, Sr^{2+} and Ba^{2+} complexes on the other possibly arises due to a difference in the structure of these complexes formed as a direct consequence of the difference in ionic radii of these metal ions. Metal ions with smaller ionic radii such as Mg^{2+} (0.66 Å) and Co^{2+} (0.72 Å) exhibit larger $\Delta[\theta]_{max}$ values at 290 nm than Ca^{2+} (0.99 Å), Sr^{2+} (1.12 Å) and Ba^{2+} (1.34 Å). Since the large positive 290 nm band corresponds to the carbonyl region, both Mg^{2+} and Co^{2+} probably coordinate to the carbonyl oxygen more effectively than they do with the carboxylate oxygen and benzoxazole nitrogen. In biological membranes only 1 : 1 complexes with Ca^{2+}, Mg^{2+} and Mn^{2+} are detected [14]. ESR studies have also shown that a mixture of 2 : 1 and 1 : 1 complexes are readily formed with Cu^{2+} in 90% ethanol [15].

It is interesting to note that Cu^{2+} and Mn^{2+} like Mg^{2+} and Co^{2+} have ionic radii of 0.72 and 0.8 Å, respectively, implying that the smaller the cation, the more probable it is for a mixture of 2 : 1 and 1 : 1 complexes to be formed at high concentrations of metal ions. Also, the small size of Mg^{2+} (and Co^{2+}) can favour only a regular octahedral complex with Mg^{2+} exhibiting a coordination of six, whereas the other divalent metal ions like Ca^{2+}, Sr^{2+} and Ba^{2+} can extend their coordination number by accepting one or more solvent molecules. The calcium complex of this ionophore exhibits a coordination number of seven [6].

Unlike divalent metal ions, lanthanides absorb in the ultraviolet/visible region. Lanthanide salts cannot exhibit CD spectra. Also, there is no literature available at present to indicate that the lanthanide metal ions exhibit CD spectra due to induction by ligand molecule. Hence, the complicated CD bands observed in the presence of the lanthanides probably arise exclusively from the ionophore molecule.

It was shown by CD titration and Scatchard plots that the lanthanide metal ions form 2 : 1 complexes. If a lanthanide metal ion binds to three ionophore molecules a ρ value of 0.66 would have been obtained, But a ρ value of only 0.5 was observed, indicating that disposition of three ligand molecules around a small metal ion of lanthanide size may not be possible. Here also two ligand molecules coordinate to the metal ion through the carboxylate oxygen, carbonyl oxygen and benzoxazole nitrogen, but the geometries of orientation of the three ligand groups are different from those of the divalent metal ions. While La^{3+}, Yb^{3+} and Eu^{3+} show different geometry of disposition of the carbonyl group from that of the divalent metal ions (molar ellipticity values observed at 320 nm), Yb^{3+} exhibits a further difference (from La^{3+} and Eu^{3+}) in the disposition of the benzoxazole nitrogen region (as inferred from the shift of the 270 nm band to 303 nm). These metal ions may also contain additional solvent molecules (at least three) coordinated to them besides the two ionophore molecules, since they can extend their coordination number to nine and above. Differences in the structure of the complexes between the divalent metal ions and lanthanide metal ions probably arise because of the large charge-to-size ratio observed for the latter and the different coordination chemistry observed with these two types of metal ions. For the same charge-to-size ratio obtained for Mg^{2+} (3.03) and La^{3+} (2.95), different CD spectra are obtained between La^{3+} and Mg^{2+} because of the difference in their coordination chemistry.

CD titration and Scatchard plots indicate that the stoichiometries of the complexes formed in the presence of Mg^{2+}, Co^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} and La^{3+} are a mixture of 2 : 1 and 1 : 1. Since the stability constant values for the 1 : 1 complexes are very low, in the presence of low concentrations of metal ions predominantly 2 : 1 complexes only are formed. Generally, the binding constant values determined for the 2 : 1 complexes of the divalent metal ion decrease as the ionic radii increase (table 3). However, certain anomalies are also found. Co^{2+}, whose ionic radius (0.72 Å) is slightly greater than that of Mg^{2+} (0.66 Å) has a lower binding constant value than Ca^{2+} (0.99 Å). Also, La^{3+} (1.016 Å) and Eu^{3+} (0.95 Å) exhibit much lower values than Ca^{2+}. The values for the divalent metal ions agree with their relative stability constants obtained from organic phase extraction techniques [1].

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