ratio of syn to anti in the presence of boric acid is much lower than the ratio in the presence of the other catalysts. Second, while the pH changes during the dehydration reaction in the presence of boric acid, there is no pH change in the presence of the other buffers. Third, when methoxyamine is the nucleophile, there is no pH change during the addition or dehydration reaction in the presence of boric acid, and the ratio of syn to anti is 0.84, which is about the same value obtained for hydroxylamine for all buffers but boric acid. Fourth, boric acid catalysis is more pronounced for hydroxylamine than for methoxyamine. Fifth, in the concentration range 0.2 to 0.5 M for boric acid, k_{obsd} vs. concentration is linear for methoxyamine but nonlinear for hydroxylamine. Since the methyl group of methoxyamine can block the formation of the cyclic compound, these results are consistent with the suggested mechanism. In principle, the nonlinear dependence of k_{obsd} for hydroxylamine can be fit to a rate law which accounts for the formation of the cyclic compound and general acid catalysis by boric acid. However, the reaction is complicated by additional equilibria involving polymers of boric acid,[13] as indicated by the fact that the k_{obsd} vs. concentration dependence becomes nonlinear for methoxyamine when the concentration of boric acid is less than 0.1 M. A unique fit of the data in Figure 5 by including parameters for these equilibria, as well as the other parameters, becomes questionable in view of the number of variables that would be involved. Consequently no fit was made.

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(14) All concentrations for hydroxylamine and the various buffers are reported as total concentrations, including all states of protonation.
(15) Under these conditions, the CH_3 resonance of the aldehyde is not observed and the CH_3 resonance of the carbinolamine is not broadened measurably compared with the case in which the base is in excess. In addition, the CH_3 resonance of the carbinolamine shifts down field as the initial concentration of aldehyde increases relative to the base concentration. These results indicate that the equilibrium between aldehyde and carbinolamine is rapid on the nmr time scale. Other examples in which this is not the case will be reported in subsequent papers.
(16) In those studies, information about the dehydration step was obtained by measuring the equilibrium constant for the formation of the carbinolamine as well as the rate of hydrazone formation.

Crystal and Molecular Structure of the Calcium Ion Complex of A23187

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Abstract: The antibiotic A23187 is a monocarboxylic acid which has been shown to bind and transport divalent cations across natural and artificial membranes. The 1:2 calcium ion complex of the ionophore A23187 crystallizes in space group $C2$ with cell dimensions $a = 23.715$ (7), $b = 15.130$ (4), and $c = 17.841$ (6) Å, $\beta = 90.67$ (4)°, and $Z = 4$. The structure has been refined to a residual of 0.11. The coordination number of the calcium ion is seven. Each ionophore is bound to calcium through a carboxyl oxygen, a carbonyl oxygen, and the nitrogen of the benzoxazole ring system. The seventh position is occupied by a water molecule. Head-to-tail hydrogen bonding is observed between the two A23187 ions of the complex.

The antibiotic A23187 is a monocarboxylic acid which has been shown to bind and transport divalent cations across natural and artificial membranes.[1,2] By equilibrating calcium ion across the inner membrane of mitochondria, A23187 can uncouple oxidative phosphorylation.[3] Various biological processes which require the presence of calcium ion, such as thyroid secretion,[4] insulin release,[5] and the simulation of the action of epinephrine on the α-adrenergic receptor,[6] have been stimulated using this ionophore. Because of the fluorescent properties of A23187, it has been used also as a probe for divalent cations in artificial and biological membranes and to determine the mode of action of ionophore-mediated divalent cation transport.[2] The structure of the free acid of A23187 has been determined by chemical methods and x-ray crystallography[7] and has been shown to be that illustrated in Figure 1. We report here the crystal structure of the 1:2 calcium ion complex of the ionophore.

Experimental Section

Single crystals of the calcium ion complex of A23187 are parallelepipeds and were grown from a 95% ethanol solution containing the ionophore and calcium acetate. A single crystal measuring 0.6 × 0.6 × 0.5 mm was mounted in a glass capillary. Pertinent unit cell data are given in Table I. The intensities of 9605 independent reflections (sin $\theta/\lambda \leq 0.70$ Å^{-1}) were measured on an Enraf-Nonius CAD-4 diffractometer using zirconium-filtered molybdenum radiation. No significant changes were observed in the intensities of two standard reflections which were measured after every 96 intensities were recorded. Intensities were corrected for Lorentz and
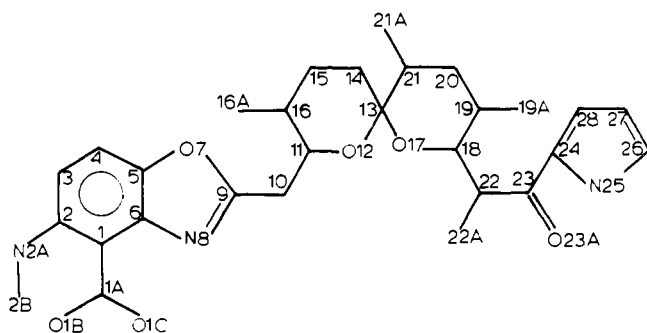
Figure 1. Carbon skeleton of A23187 with the numbering system used in this study.

Table I. Pertinent Unit Cell Data for $Ca(C_{29}H_{36}N_3O_6)_2 \cdot 2H_2O$

$a = 23.715\ (7)$ Å	
$b = 15.130\ (4)$ Å	$\beta = 90.67\ (4)°$
$c = 17.841\ (6)$ Å	
$Z = 4$	Space group $C2$
$d_o = 1.18$ g cm^{-3}	$d_c = 1.16$ g cm^{-3}
μ (Mo Kα) = 1.62 cm^{-1}	

polarization factors but not for extinction or absorption. Atomic scattering factors were taken from the literature; both real and imaginary dispersion corrections were applied.[8] On the basis of a $2\sigma(I)$ test, 2840 data were considered observed. The variance of each F was calculated according to the method of Stout and Jensen[9] $[\sigma^2(F) = k/4(Lp)I[\sigma^2(I) + (0.06I)^2]; w(F) = 1/\sigma^2(F)]$. Unobserved data were given zero weight and not included in the refinement.

Structure Determination and Refinement. Through the use of MULTAN,[10] positional parameters were obtained for 38 atoms. Three cycles of Fourier refinement revealed the positions of the remaining 40 non-hydrogen atoms. The structure was refined isotropically by full-matrix least-squares minimizing $\Sigma w\Delta^2$ to a residual of 0.11 ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$) and a weighted residual of 0.15 $[R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2}]$. S, the standard deviation of an observation of unit weight, was calculated to be 3.1 $[S^2 = \Sigma w\Delta^2/(m - n)$ where m is the number of observations and n is the number of parameters]. The absolute configuration, although not determined, has been assigned to correspond to that of the free acid form.[7,11]

Bond distances and angles within both A23187 ions are unexceptional.[11] The coordination number of calcium is seven. Each A23187 ion is bonded to calcium through one oxygen of the carboxyl group and through the oxygen of the carbonyl group; this coordination of oxygen is approximately square-planar. The nitrogen atoms of the benzoxazole ring system are also coordinated to the calcium ion. These nitrogen atoms, together with the calcium ion, lie in a plane nearly perpendicular to the plane of the four oxygen atoms and almost bisect the carbonyl oxygen-calcium-carboxyl oxygen angles of each ionophore. The remaining apical position is occupied by a water molecule. Distances and angles involving the coordination of calcium ion are summarized in Table II. The observed conformation of the 1:2 complex is illustrated in Figures 2 and 3. The complex possesses pseudo-twofold symmetry, the twofold axis being coincident with the bond between the water molecule and the calcium ion.

Only one internal hydrogen bond is observed in each A23187 ion; the nitrogen of the secondary amino group is hydrogen bonded to an oxygen of the carboxyl group. A second hydrogen bond exists between the nitrogen of the pyrrole belonging to one A23187 ion and the carboxyl group of the other ion in the complex. These hydrogen bonds are illustrated as dashed lines in Figure 2. Thus interionic head-to-tail hydrogen bonding helps keep the complex in a fairly rigid conformation.

The second water molecule, located from a Fourier map, has rather high thermal motion and may have only partial occupancy. It is held by hydrogen bonds from the water molecule bound to calcium and to a carboxyl oxygen of a neighboring complex.

Table II. Distances (Å) and Angles (deg) Involving Calcium Ion along with Their Estimated Standard Deviations

Bond Distances			
Ca–O (1C1)	2.27 (1)	Ca–O (23A1)	2.37 (1)
Ca–O (1C2)	2.28 (1)	Ca–O (23A2)	2.38 (1)
Ca–N (81)	2.69 (1)	Ca–O (1S)	2.38 (1)
Ca–N (82)	2.58 (1)		

Angles			
O(1C1)–Ca–N (81)	68.4 (6)	O(1C1)–Ca–N (82)	109.8 (6)
O(1C1)–Ca–O (23A1)	106.7 (6)	O(1C1)–Ca–O (23A2)	82.7 (6)
O(1C1)–Ca–O (1C2)	172.0 (6)	O(1C1)–Ca–O (1S)	85.5 (7)
O(1C2)–Ca–N (81)	118.9 (6)	O(1C2)–Ca–N (82)	70.6 (6)
O(1C2)–Ca–O (23A1)	79.0 (6)	O(1C2)–Ca–O (23A2)	89.8 (6)
O(1C2)–Ca–O (1S)	90.5 (7)	N(81)–Ca–N (82)	77.0 (6)
N(81)–Ca–O (23A1)	75.9 (6)	N(81)–Ca–O (23A2)	130.2 (6)
N(81)–Ca–O (1S)	134.5 (7)	N(82)–Ca–O (1S)	148.5 (7)
N(82)–Ca–O (23A1)	121.4 (6)	N(82)–Ca–O (23A2)	76.0 (6)
O(23A1)–Ca–O (23A2)	153.4 (6)	O(23A1)–Ca–O (1S)	76.9 (7)
O(23A2)–Ca–O (1S)	79.1 (6)		
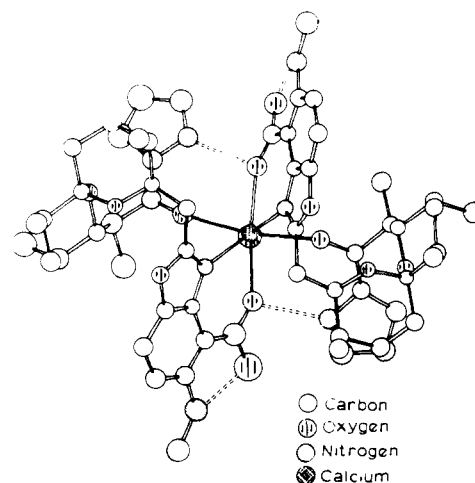
Figure 2. Calcium ion complex of A23187 observed along the bond between calcium and the water molecule. Bounding spheres are drawn at the 30% probability level.
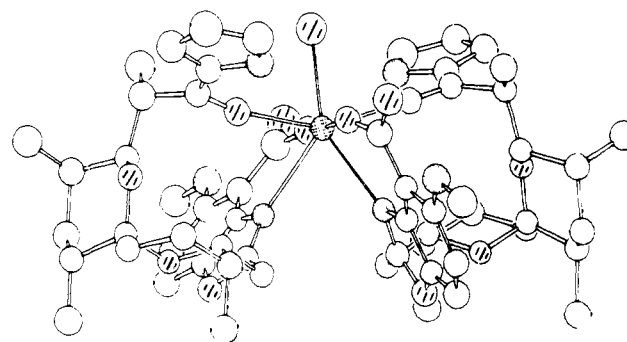
Figure 3. Calcium ion complex of A23187 viewed perpendicular to the plane containing the calcium ion and the two nitrogens bound to calcium. Bounding spheres are drawn at the 30% probability level.

Discussion

Because of the rigidity of the spiro ring system and the π bonding in the pyrrole and benzoxazole ring systems, there

Figure 4. Superposition of one A23187 ion (dark lines) on one molecule of the free acid form[7] (light lines). The spiro ring systems in both drawings have identical orientations.

Table III. Torsion Angles Involving the sp^3 Carbon Atoms Adjacent to the Spiro Ring System for the Complexed and Free Acid Form[7]

		Ion 1, deg	Ion 2, deg	Free acid form, deg
χ_1	O(7)–C(9)–C(10)–C(11)	−84	−96	132
χ_2	N(8)–C(9)–C(10)–C(11)	90	80	−46
χ_3	C(9)–C(10)–C(11)–O(12)	51	56	174
χ_4	C(9)–C(10)–C(11)–C(16)	176	178	−65
χ_5	O(17)–C(18)–C(22)–C(23)	62	57	66
χ_6	C(19)–C(18)–C(22)–C(23)	−178	179	−172
χ_7	C(18)–C(22)–C(23)–C(24)	−116	−96	−158

are only four bonds in each A23187 ion which are capable of any significant amount of conformational flexibility. These bonds occur in pairs on either side of the spiro ring system and involve one sp^3-hybridized carbon each. Torsion angles involving these sp^3 atoms are listed in Table III for both ions, along with the corresponding values obtained from the crystal structure determination of the free acid form of A23187.[7] Differences of as much as 20° in the torsion angles are observed between the two crystallographically independent ions in the complexed form. However, when the torsion angles of the complexed form are compared to those of the free acid form, the change in angle becomes as large as 138°. The effect of these changes in torsion angle on the gross conformation is illustrated in Figure 4 where one ion of the complexed form and one molecule of the free acid form are superimposed. In both cases, the spiro ring system has been given the same orientation and the change in position of the pyrrole and benzoxazole ring systems may be clearly seen.

One very interesting feature of this structure is the overall shape of the complex, as seen in Figure 3. The oxygen atoms of the spiro ring system are located in the central portions of each ion and are shielded from the environment. The surface of one side of the complex is composed mainly of hydrogen atoms; the other side of the complex containing the calcium ion has, in exposed positions, atoms capable of donating or accepting a hydrogen bond. With the exception of the water molecule bonded to calcium, this side of the complex is rather flat.

The overall structure of the complex has some features in common with that of the lithium ion complex of antaminide.[12] In this latter case, the folding of the decapeptide ring forms a cup in which the lithium ion is located. The floor of the cup is comprised of four carbonyl oxygen atoms which form a square-planar array about the lithium ion.

The fifth ligand is an acetonitrile solvent molecule which sits above the shallow cavity of the cup. In the present study, the calcium ion is nearly in the plane of the rim of the "cup"; in addition, the cup has holes on opposite sides and no bottom. Unlike the calcium ion complex of A23187, the surface of the antaminide complex is almost completely hydrophobic.

Supplementary Material Available: listing of atomic coordinates, bond distances and angles, and a tabulation of observed and calculated structure factors (20 pages). Ordering information is given on any current masthead page.

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