SEMI-SYNTHESIS OF A23187 (CALCIMYCIN) ANALOGS

III. MODIFICATION OF BENZOXAZOLE RING SUBSTITUENTS, IONOPHOROUS PROPERTIES IN AN ORGANIC PHASE

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Ten semi-synthetic analogs of A23187 (calcimycin), with only the benzoxazole ring substituents modified together with the ionophore X14885A were studied with regard to their calcium and magnesium carrier properties through an organic phase (toluene - butanol, 70 : 30). The results indicate that the carboxylic group and the oxazolic nitrogen, maintained in the *ortho* position are essential for the ionophorous properties. Further, the introduction of a substituent in place of the $NHCH_3$ group, producing steric hindrance of the carboxylic group leads to a destabilization of the 2 : 1 associations with cations.

A23187 (calcimycin, **1**) is an antibiotic isolated from a strain of *Streptomyces chartreusis* (NRRL 3882)[1] and belongs to the important family of carboxylic polyether ionophores[2]. Its structure has been shown to be specifically suited to the transport of alkaline-earth cations through membrane phases *via* 2 : 1 neutral complexes[3]. Since its discovery, this ionophore has been widely used as a tool for investigating the role of calcium in biological systems[4], and more recently for synergistic effects with active compounds such as phorbol esters[5].

X-Ray crystallographic studies of the calcium[6] and magnesium[7] salts revealed that the 2-carboxy-3-*N*-methylaminobenzoxazole ring is the main cation binding site in the $(A23187)_2 : M^{++}$ complexes (Fig. 1). In addition to ionic charge neutralization, one oxygen of the carboxylic group and the pyridine-like nitrogen of the oxazole ring are involved in ligand formation with the divalent cation.

In order to determine the respective roles of the substituents fixed on the benzoxazole moiety, we carried out chemical modifications to the naturally-occurring structure. From a selectively cleaved calcimycin, we have worked out a semi-synthetic approach in several steps which provides analogs with the correct overall stereochemistry[8,9]. In this paper, the work is completed by the synthesis of two new compounds **6** and **7** bearing a methyl and a hydroxyl group respectively in place of the 3-*N*-methylamino group. In addition, we report the preparation of derivatives **9**, **10**, **11** obtained from calcimycin by chemical modifications of the *N*-methyl group.

The set of compounds **1**~**11** made available in this way have provided us with an insight into the divalent cation carrier properties of suitably designed structures. We have included in our study the divalent ionophore X14885A (**12**) recently isolated by WESTLEY *et al.*[10]. All the structures investigated are shown in Table 1.

Chemistry

Synthesis of Compounds 6 and 7

These two analogs were obtained by the reactions described in Scheme 1.

The degradation of **1** to the carboxylic acid **21** has already been described[8] and will not be further

Fig. 1. Calcium and magnesium coordination sphere in the $(A23187)_2$: M^{++} complexes, following ALLEAUME and BARRANS representation[7].

The oxygen and nitrogen coordinating atoms are represented by ○; for >C(20)=O, ●; for ⩾C(1)–O⁻ of the carboxylate, ▶; for the oxazolic nitrogen, repeated twice in the dimeric association of A and A′ molecules.

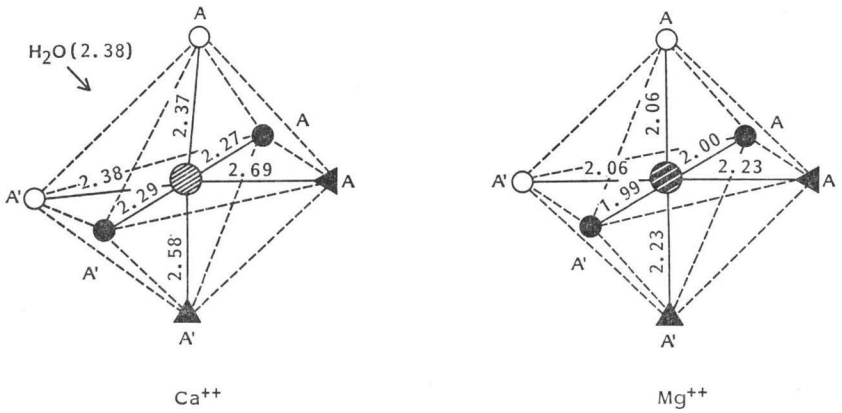
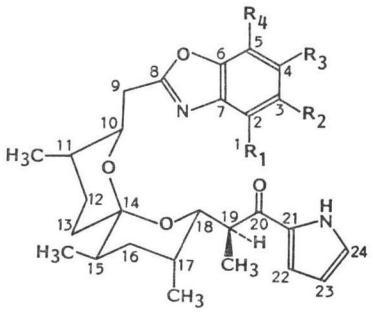


Table 1. Analogs studied.
Numbering is that of calcimycin (**1**).

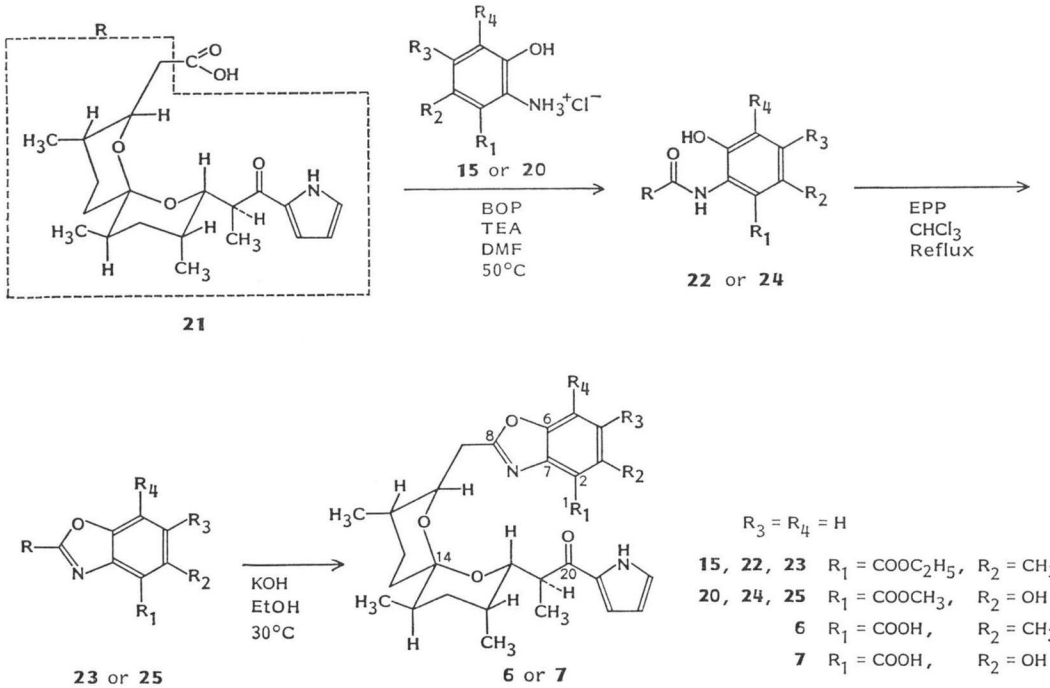


Compounds	R_1	R_2	R_3	R_4
1	COOH	$NHCH_3$	H	H
2	H	COOH	H	H
3	COOH	H	H	H
4	COOH	H	H	CH_3
5	COOH	H	CH_3	H
6	COOH	CH_3	H	H
7	COOH	OH	H	H
8	COOH	$N(CH_3)_2$	H	H
9	COOH	$N(CH_3)C_2H_5$	H	H
10	COOH	$N(CH_3)COCH_3$	H	H
11	COOH	$N(CH_3)COCF_3$	H	H
12*	COOH	OH	H	H

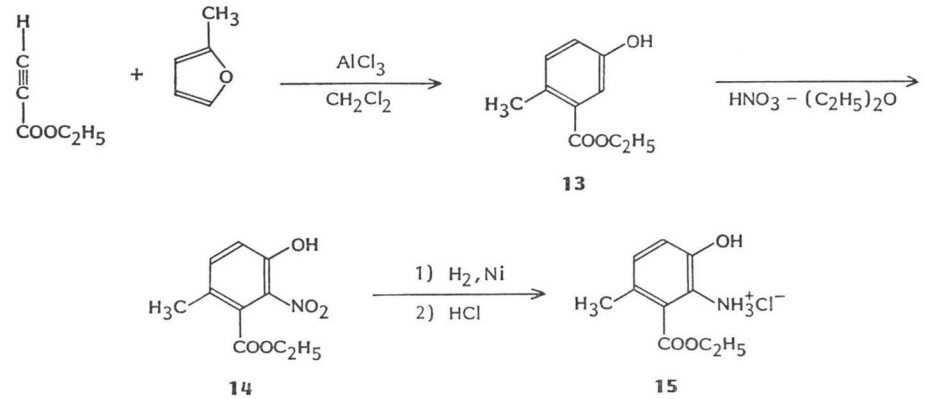
* X14885A: Backbone without 15-methyl.

discussed. Synthesis of **6** (R_1=COOH, R_2=CH_3, R_3=R_4=H) required the preparation of a 3-hydroxy-6-methyl anthranilate ester. We had failed previously to obtain this intermediate by rearrangement of the appropriate free hydroxylamine with TsCl - NEt_3[9]. This synthesis was achieved *via* another route as shown in Scheme 2.

Scheme 1.



Scheme 2.

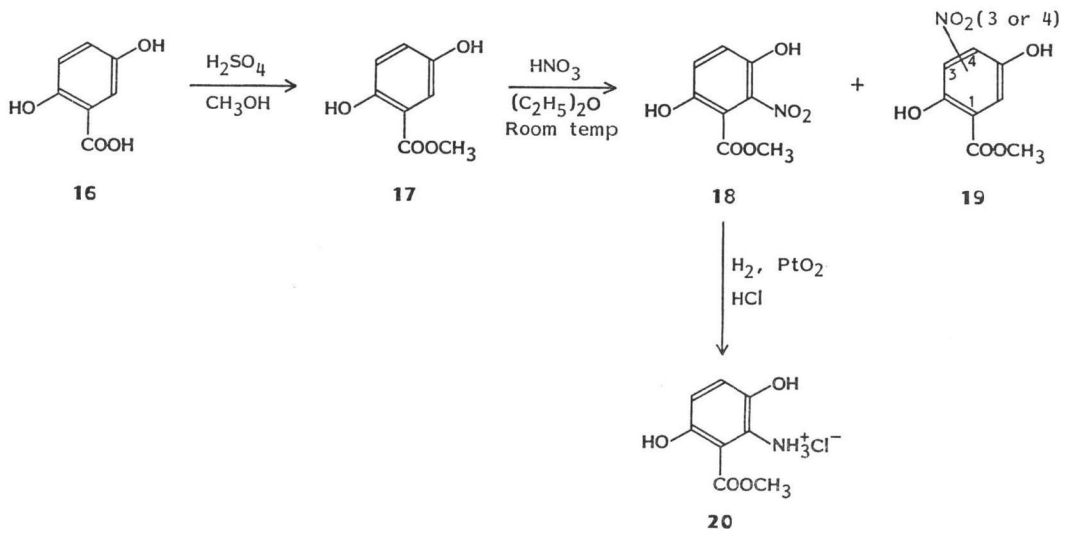


A Diels-Alder reaction between ethyl propiolate and 2-methylfuran led to the ester **13**[11] which was separated from by-products by a sequence of selective extractions. This compound was nitrated to give **14** as the major product, the reduction of which, over Raney nickel, yielded **15**.

The structure of compound **7** is very close to that of X14885A (**12**), differing only by a methyl added in the 15-position on the spiroketal group. The preparation of the benzoxazole precursor **20** was carried out starting from gentisic acid (**16**) according to Scheme 3.

The two hydroxyanthranilates **15** and **20** were respectively coupled with the synthon **21** (Scheme 1) with the help of benzotriazolyl *N*-oxytrisdimethylaminophosphonium (BOP) reagent[12], cyclization

Scheme 3.



to the oxazoles being accomplished with ethyl polyphosphate (EPP)[13]. The hydrolysis of the ester group gave the calcimycin analogs **6** and **7**.

In the case of the *p*-diphenol **20**, it was necessary to carry out coupling reaction under nitrogen to minimize air oxidation. However, the cyclization of the intermediate amide with EPP occurred rapidly.

Synthesis of Compounds **9**, **10** and **11**

N-Ethyl (**9**), *N*-acetyl (**10**) and *N*-trifluoroacetyl (**11**) calcimycins were obtained by conventional reactions performed on the natural metabolite, under the conditions described below.

We studied the conformation of the twelve calcimycin analogs by ^1H NMR at 400 MHz, in both chloroform and methanol. Careful examination of the coupling constants led us to the conclusion that all the compounds adopted almost identical conformations corresponding to a closed structure in chloroform with a head-to-tail chelation and a more open structure in methanol where the methylene benzoxazole arm is rotated. These results will be described in detail elsewhere.

Calcium and Magnesium Carrier Properties

It is evident from reviews dealing with this problem[14], that there is no single test to characterize a carboxylic polyether ionophore. On the basis of both previous work done mainly by PFEIFFER and co-workers on calcimycin[15] and our own experimentation, we chose several complementary methods to obtain a characterization that was as complete as possible, purely physico-chemical measurements are described in this paper.

The commonly accepted overall transport process for calcimycin is a M^{++}-$2H^+$ antiport, represented in Fig. 2.

As we have shown in a model triphasic cell (water - chloroform - water)[16], the cation extraction is dependent on the acidic dissociation of the carrier and the heterogeneous formation constants of the monomeric and dimeric complexes at the interface. Further, during the release step we observed recently[17] that the kinetics are very different for calcium and magnesium, which may explain the much higher ionic flux for calcium in all membrane phases. These general considerations underly the choice of the physico-chemical methods used, the results of which are collected in Tables 2 and 3.

Fig. 2. Calcimycin: a M^{++}-carrier by an antiport M^{++}-$2H^+$ mechanism.

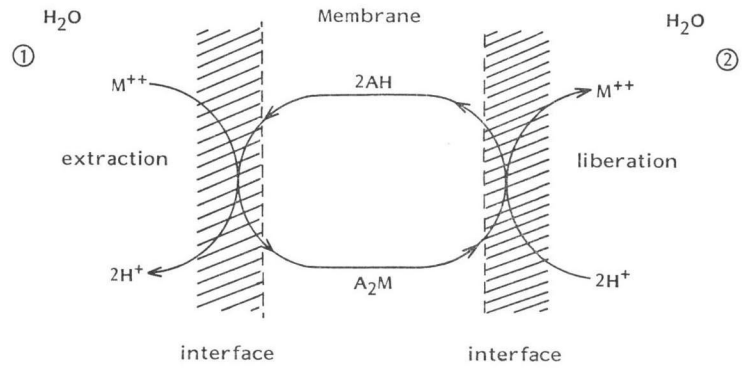


Table 2. Physico-chemical characterization of the analogs.

pKa, overall extraction constants β_1 and relative intrinsic extraction $\Delta \log \beta_2$ in a H_2O - toluene-butanol (70 : 30) two-phase system.

Compounds	*pKa* (MeOH - H_2O, 70 : 30)	$-\log \beta_1$		$\Delta \log \beta_2$	
		Ca^{++}	Mg^{++}	Ca^{++}	Mg^{++}
1	7.30	6.4	6.9	0	0
2	6.34	13.3	12.7	8.8	7.7
3	6.02	5.1	5.9	1.2	1.6
4	6.53	6.9	6.9	2.0	1.5
5	6.04	5.1	5.1	1.2	0.7
6	5.19	7.7	8.1	4.5	5.4
7	4.40	1.7	2.3	1.0	1.2
X14885A (**12**)	4.48	1.7	2.3	0.9	1.1
8	7.10	9.4	10.3	3.4	3.8
9	7.10	10.3	11.3	4.3	4.8
10	4.95	5.5	6.9	3.8	4.7
11	4.87	5.3	6.3	3.7	4.2

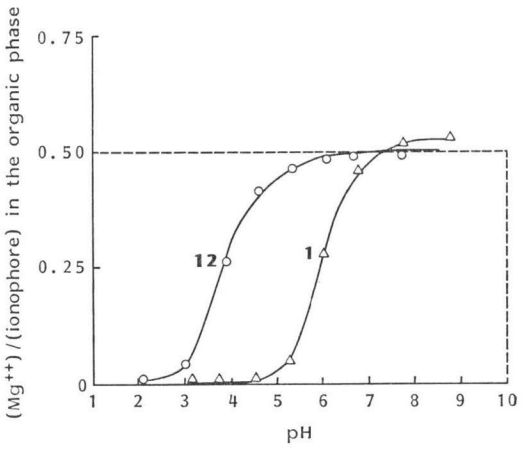
Acid Dissociation: The *pKa* values given in Table 2 were measured by UV spectrophotometry according to KAUFFMAN *et al.*[18], in methanol - water, 70 : 30 (w/w), which has been proposed as a possible model for the membrane-water interface for the study of carboxylic polyether dissociation[19]. The values range over 2.9 *pKa* units. Compounds **7** and **12** with an OH group in the 3 position are the most easily dissociated, as expected for a salicylic group, in contrast calcimycin (**1**) is the least acidic system. For other molecules, the acidity constants are the reflection of steric and electronic effects of substituents acting both on the acid and the anionic forms; this is especially the case when R_2 is a bulky substituent for **6** and **8** to **11**.

Ionic Exchanges in a Two-phase Extraction System: This technique has been used for the characterization of ionophorous molecules giving neutral[20] or charged complexes[21]; the organic phases have varied however. Calcimycin has been studied in detail using the water - toluene-butanol, 70: 30 system[22]; accordingly we chose this system for the sake of consistency.

Typical extraction curves where the ratio (M^{++})/(ionophore) in the organic phase is plotted versus the aqueous phase pH are shown in Fig. 3 for the naturally-occurring compounds **1** and **12** which are at the two extremes of the *pKa* scala. As already pointed out[16,22], the extraction is clearly pH-dependent which indicates a proton/metal-ion competition; the asymptote value near 0.5 corresponds to the

Fig. 3. Mg^{++} extraction curves for **1** and **12** in a biphasic system: H_2O ($(M^{++})=5\times10^{-2}$ M, variable pH)/toluene - butanol, 70: 30 ((ionophore)= 10^{-4} M).

1; pKa=7.30, **12**; pKa=4.48.



formation of a neutral dimeric complex. Results for calcimycin are in good agreement with previous ones[22].

All the twelve compounds studied gave the same kind of sigmoidal curves but distinctly shifted either towards the alkaline pH region for **2, 6, 8** and **9** or towards the acid region for **7, 10, 12.** In this effect both the acid dissociation and the extracting capacity for each compound are involved. We have attempted to estimate the extent to which each of these factors is implicated.

The heterogeneous equilibrium, which can be studied experimentally, can be written.

$$2AH_{organic}+M^{++}{}_{aq} \rightleftharpoons A_2M_{organic}+2H^{+}{}_{aq} \quad (1)$$

where AH stands for the protonated form of the ionophore.

The corresponding β_1 constants, calculated at the point of half-saturation as previously proposed[22], are given in Table 2 for compounds **1**~**12.**

When, for instance, the naturally-occurring metabolites **1** and **12**, are compared, there is a large difference in extracting capacity in favor of the latter, as shown by the β_1 values. This is at variance with the results obtained in methanol for the homogeneous equilibrium:

$$2A^{-}{}_{MeOH}+M^{++}{}_{MeOH} \overset{\beta'_2}{\rightleftharpoons} A_2M_{MeOH} \quad (2)$$

where the respective log β'_2 values are: for **1**, Ca^{++}; 16.2, Mg^{++}; 15.9[23]: for **12**, Ca^{++}; 15.9, Mg^{++}; 14.9 (unpublished work), *i.e.* of the same order of magnitude and in fact higher for calcimycin. This discrepancy is due to the fact that the acid dissociation (3) is included in the overall equilibrium.

$$AH_{organic} \rightleftharpoons A^{-}{}_{interface}+H^{+}{}_{aq} \quad (3)$$

$$2A^{-}{}_{interface}+M^{++}{}_{aq} \overset{\beta_2}{\rightleftharpoons} A_2M_{organic} \quad (4)$$

As the pKa at the interface is not known the β_2 value cannot be calculated. However, assuming that the differences between the pKa given in Table 2 remain the same at the water - toluene-butanol interface, an increment value can be calculated.

$$\Delta \log \beta_2=\Delta \log \beta_1+2\,\Delta pKa_{(\text{methanol - water, 70:30})}$$

with respect to calcimycin (**1**), for instance. Results obtained in this way are given in Table 2. They may reflect more accurately the intrinsic complexing properties of the molecules. All the differences are positive, and so **1** proves to be the best extracting system for calcium and magnesium but **12, 7** and then **5, 3** and **4** are not far behind. Structures where R_2 is a bulky substituent, hindering the carboxylic group, and in which there is no longer any possibility of hydrogen bonding with this group, are poorer complexing systems, *e.g.* **6** (*ortho*-CH_3) and **8** to **11** (*N*-methyl substituted). Not unexpectedly when $R_1=R_3=R_4=H$ and $R_2=COOH$ the corresponding structure **2** loses all the calcimycin properties. The different situation with unfavorable steric arrangements are shown in Scheme 4.

Scheme 4.

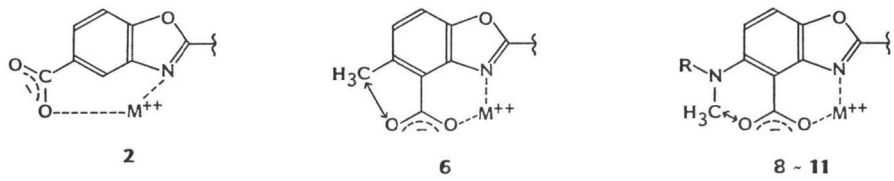


Fig. 4. Release curves for **1** and **12**.
pH of the aqueous phases are mentioned on the curves.
○ Ca^{++}, ● Mg^{++}.

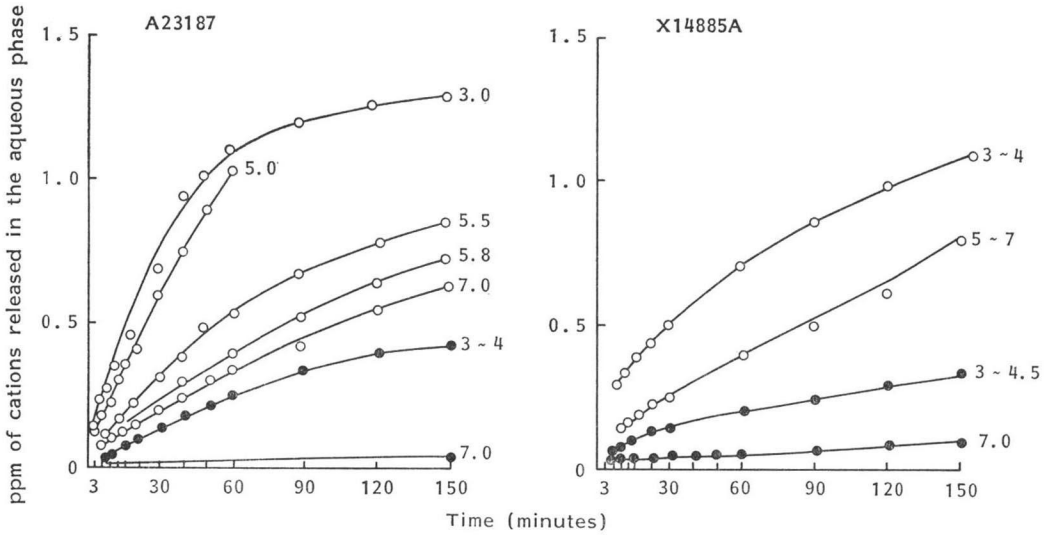


Table 3. Initial rates of decomplexation (ppm × mn^{-1} × 10^3).

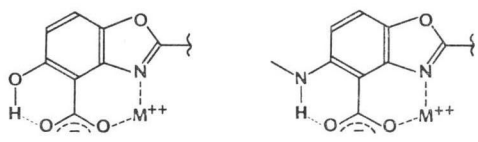
Compounds	Ca^{++}			Mg^{++}	
	pH 7	pH 5	pH 3	pH 7	pH 3 or 4
1	8.6	29	46	0.3	5
3	85	85	183	5.3	18.3
X14885A (**12**)	14	14	37	2.1	8
8	95	10.3	15.3	28	46
9	126	19	35	15	11
10	116	116	116	28	17
11	60	60	128	6	16

The first conclusion emerging from these results is the prominent role of the 2-carboxyl benzoxazole sequence in the formation and stability of complexes.

No marked selectivity was evident between calcium and magnesium in the extraction experiments. For the reasons given above we made kinetic measurements for the decomplexation step, in the same two-phase system.

Decomplexation Kinetics in a Two Phase System: The study of the overall kinetics of cation release in a water - toluene-butanol (70: 30) system was technically straightforward (see Experimental part), provided the 2 : 1 neutral complex initially introduced into the organic phase was carefully monitored. Typical release curves obtained for **1** and **12** are shown in Fig. 4, at different pH of the aqueous

Scheme 5.



phase. Initial rates of decomplexation in ppm $\times mn^{-1} \times 10^3$ were calculated for compounds available in sufficient quantity. Results are collected in Table 3.

For all the compounds tested in this organic phase, the magnesium release is systematically slower than that of calcium. Therefore, by analogy with calcimycin, a higher transmembrane flux for calcium is expected, although the extraction constants (which are the ratio of complexation rate to decomplexation rate) are of the same order of magnitude. This could be explained by the structural differences existing between Ca^{++} and Mg^{++} complexes and also by different kinetics of rehydration for the cations[24].

The marked stability of associations with **1** and **12** is shown by the low initial rates measured. This can be ascribed to the hydrogen bonding network giving an organized structure, as shown in Scheme 5.

Further, for calcimycin, the initial rates of release were pH-dependent, due to the protonation of the secondary amine site over the acidic range.

Thus, these physico-chemical results obtained in an organic phase throw a new light on the complexation mechanism in the benzoxazole region and the Ca^{++}/Mg^{++} selectivity. But, this highly simplified model does not necessarily mimic biological membrane behavior. This comparison is undertaken in the following paper.

Experimental

NMR spectra were recorded on a Perkin-Elmer R24 (^{1}H NMR, 60 MHz) for routine studies or on Bruker spectrometers (WP 200 and WM 400) for high field spectra, with tetramethylsilane as internal standard. The resonance values are expressed in parts per million (δ). EI mass spectra were determined with either VG. 70–70 F or VG. 30 F spectrometer. The exact mass was measured when indispensable sample drying for C, H, N analysis was difficult to achieve (small amounts, decomposition . . .). Optical rotations were measured with a Perkin Elmer model 141 polarimeter. Melting points were determined on a Reichert hot plate apparatus and are uncorrected TLC analysis was performed with Schleicher and Schüll plastic silica gel plates (F 1500/LS 254), home-made glass plates with Merck Kieselgel (60 PF 254–366) were used for the preparative scale. Column chromatography was carried out using Merck Kieselgel (60/70 230 mesh A STM). A23187 (calcimycin, **1**) was from the stock sample of our laboratory, as was X14885A (**12**) isolated recently from the strain NRRL 12350. This novel ionophore was strictly identical (UV, IR, NMR, mass) to a sample kindly provided by Dr. J. W. WESTLEY.

Synthesis

Ethyl 3-Hydroxy-2-nitro-6-methylbenzoate (**14**)

Compound **13**[11] (3.8 g) was stirred for 1 hour at $-20°C$ in an ether - fuming nitric acid solution (21 ml, 20 : 1). The resulting mixture was separated on a column (silica gel, eluent; cyclohexane - EtOAc, 50: 50). The first fraction eluted (150 mg, yellow solid) was identified as a 4- or 5-mononitrobenzoate and discarded: ^{1}H NMR (60 MHz, $CDCl_3$) δ ppm/TMS 1.35 (3H, t, $COOCH_2CH_3$), 2.2 (3H, s, Ar-CH_3), 4.3 (2H, q, $COOCH_2CH_3$), 7.8 and 8.2 (2H, 2×s, Ar). The second fraction (750 mg, yellow solid) was the 2-nitrobenzoate **14**: MP 20~25°C; ^{1}H NMR (60 MHz, $CDCl_3$) δ ppm/TMS 1.3 (3H, t, $COOCH_2CH_3$), 2.3 (3H, s, Ar-CH_3), 4.35 (2H, q, $COOCH_2CH_3$), 7.23 (2H, AB system, q, J=8.5 Hz, Ar). Third fraction (246 mg, yellow solid) was the 2,4- or 2,5-dinitrobenzoate: ^{1}H NMR (60 MHz,

$CDCl_3$) δ ppm/TMS 1.35 (3H, t, $COOCH_2CH_3$), 2.2 (3H, s, Ar-CH_3), 4.35 (2H, q, $COOCH_2CH_3$), 7.9 (1H, s, Ar).

Ethyl 2-Amino-3-hydroxy-6-methylbenzoate Hydrochloride (**15**)

A mixture of **14** (750 mg), abs EtOH (35 ml), Raney Ni (equiv 1 g), was shaken under hydrogen pressure (700 g/cm^2), at room temp for 2 hours. The catalyst was filtered off and the solvent removed. The residue was dissolved in dry ether and saturated with hydrogen chloride, to give **15** as a white solid (695 mg): ^{1}H NMR (60 MHz, DMSO) δ ppm/TMS 1.25 (3H, t, $COOCH_2CH_3$), 2.2 (3H, s, Ar-CH_3), 4.25 (2H, q, $COOCH_2CH_3$), 6~7 (3H, br s, NH_3^+), 6.95 (2H, AB system, q, J=8 Hz, Ar).

Methyl 2,5-Dihydroxybenzoate (**17**)

Commercial gentisic acid (10 g), MeOH (130 ml) and concd H_2SO_4 (2 ml) were heated under reflux for 12 hours. After concentration of the solution, the residue was diluted with H_2O, extracted with ether and purified by column chromatography (silica gel, eluent; cyclohexane - EtOAc, 80: 20) to give **17** (9.5 g): MP 86~87°C. The structure was confirmed by ^{1}H NMR (60 MHz, $CDCl_3$).

Methyl 2,5-Dihydroxy-6-nitrobenzoate (**18**)

17 (4 g) was treated with nitric acid as above for **14** at 0°C. Two main products were separated by column chromatography (silica gel, eluent; cyclohexane - EtOAc, 80 : 20) which were **19** (0.7 g) and **18** (2.2 g).

19 (yellow solid): ^{1}H NMR (60 MHz, $CDCl_3$) δ ppm/TMS 4.0 (3H, s, $COOCH_3$), 7.7 (2H, s, Ar).

18 (Yellow solid): MP 117~118°C; ^{1}H NMR (60 MHz, $(CD_3)_2CO$) δ ppm/TMS 3.9 (3H, s, $COOCH_3$), 7.2 (2H, AB system, q, J=9 Hz, Ar), 8.5~9.5 (2H, br s, Ar-OH); m/z (M), found 213.0297, calcd 213.0271 for $C_8H_7NO_6$.

Methyl 6-Amino-2,5-dihydroxybenzoate Hydrochloride (**20**)

A mixture of **18** (2.2 g), abs EtOH (50 ml) and PtO_2 (220 mg) were shaken under hydrogen pressure (700 g/cm^2) for 4 hours. The same method of isolation as for **15** gave **20** (1.3 g, white solid): ^{1}H NMR (60 MHz, DMSO) δ ppm/TMS 4.0 (3H, s, $COOCH_3$), 5.0 to 5.6 (3H, br s, NH_3^+), 6.5 (2H, AB system, q, J=8 Hz, Ar), 8.8~9.3 (2H, br s, Ar-OH).

Ethyl 2-*N*-((3,9,11-Trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)acetyl)-3-hydroxy-6-methylanthranilate (**22**)

A light-protected solution of **15** (100 mg) in DMF (20 ml), triethylamine (TEA, 170 mg), synthon **21** (16 mg) and benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium or BOP (200 mg), were stirred in a water-bath at 50°C for 7 hours, poured into H_2O, extracted with ether, dried over Na_2SO_4 and purified by TLC (eluent; cyclohexane - EtOAc, 50: 50) to yield **22** (150 mg) as a white foam. MP 59~60°C; $[\alpha]_{578}^{25}$ +74° (c 0.0015, $CHCl_3$); m/z (M), found 554.2976, calcd 554.2981 for $C_{31}H_{42}N_2O_7$; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 1.40 (3H, t, $COOCH_2CH_3$), 2.35 (3H, s, Ar-CH_3), 2.4 and 2.6 (2H, q, 9-H_A and 9-H_B), 4.1 (1H, m, 10-H), 4.4 (2H, q, $COOCH_2CH_3$), 6.25 (1H, m, 23-H), 6.95 (1H, br s, 22-H), 7.05 (1H, s, 24-H), 7.07 (2H, br s, Ar), 8.25, 9.4 and 9.6 (3H, br s, pyrrole NH, amide NH and OH).

Ethyl 5-Methyl-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylate (**23**)

A light-protected solution of **22** (56 mg), ethylpolyphosphate or EPP (2 g) in $CHCl_3$ (8 ml), was stirred in a water-bath at 66°C for 1 hour, diluted with H_2O, extracted with ether, dried over Na_2SO_4 to yield **23** (28 mg) as a white foam: MP 53~54°C; $[\alpha]_{578}^{25}$ +160° (c 0.0024, $CHCl_3$); m/z (M), found 536.2914, calcd 536.2876 for $C_{31}H_{40}N_2O_6$; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 1.4 (3H, t, $COOCH_2CH_3$), 2.55 (3H, s, Ar-CH_3), 2.9 and 3.1 (2H, 2×q, 9-H_A and 9-H_B), 4.05 (1H, m, 10-H), 4.5 (2H, d q, $COOCH_2CH_3$), 6.25 (1H, m, 23-H), 6.9 (1H, br s, 22-H), 7.05 (1H, br s, 24-H), 7.15 and 7.55 (2H, 2×d, Ar), 10.0 (1H, br s, pyrrole NH).

5-Methyl-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylic Acid (**6**)

A light-protected mixture of **23** (100 mg) in EtOH (100 ml) and 10% potassium hydroxide (7 ml)

was stirred at 30°C for 18 hours, poured into H_2O (200 ml), adjusted to pH 4.5 with 0.1 N HCl, extracted with ether and dried over Na_2SO_4. After ether evaporation, the residue was purified by TLC (eluent; cyclohexane - EtOAc, 50: 50). The product was then dissolved in an EtOH - H_2O - Me_2CO solution and acidified with ethanolic H_3PO_4 (10%). The solvents were removed to yield **6** (86 mg) as a white foam: MP 64~65°C; $[\alpha]_{578}^{25}$ +77° (*c* 0.0012, $CHCl_3$); *m/z* (M), found 508.2550, calcd 508.2572 for $C_{29}H_{36}N_2O_6$; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 2.8 (3H, s, Ar-CH_3), 2.93 and 3.1 (2H, 2×q, 9-H_A and 9-H_B), 4.3 (1H, m, 10-H), 6.22 (1H, m, 23-H), 6.92 (1H, br s, 22-H), 7.06 (1H, br s, 24-H), 7.29 and 7.67 (2H, 2×d, 4-H and 5-H), 9.80 (1H, br s, pyrrole NH).

Methyl 2-*N*-((3,9,11-Trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)acetyl)-3,6-dihydroxyanthranilate (**24**)

The method described for obtaining **22** from **15** was applied to **20** (100 mg) with the synthon **21**, but stirring at 50°C was maintained for 5 hours under nitrogen, giving after purification **24** (29 mg) as a white foam: MP 67~68°C. This compound was unstable and treated without further study.

Methyl 6-Hydroxy-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylate (**25**)

This compound was obtained from **24** in the same way as **23**, except that refluxing was carried out for 1 hour. White foam: MP 72~73°C; $[\alpha]_{578}^{25}$ +31° (*c* 0.0107, $CHCl_3$); *m/z* (M), found 524.2522, calcd 524.2513 for $C_{29}H_{36}N_2O_7$; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm/TMS 2.9 and 3.1 (2H, 2×q, 9-H_A and 9-H_B), 4.1 (3H, s, $COOCH_3$), 4.25 (1H, m, 10-H), 6.20 (1H, br s, 23-H), 6.90 (1H, br s, 22-H), 6.95 (1H, br s, 24-H), 7.0 and 7.65 (2H, 2×d, Ar), 10.15 (1H, br s, pyrrole NH), 11.20 (1H, s, Ar-O*H*).

6-Hydroxy-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylic Acid (**7**)

Hydrolysis of **25** (50 mg) was performed by the same method as **23** to give **7** (34 mg), white foam: MP 59~60°C; $[\alpha]_{578}^{25}$ +50° (*c* 0.023, $CHCl_3$); *m/z* (M), found 510.2355, calcd 510.2357 for $C_{28}H_{34}N_2O_7$; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 2.95 and 3.11 (2H, 2×q, 9-H_A and 9-H_B), 4.26 (1H, m, 10-H), 6.26 (1H, m, 23-H), 7.00 (1H, d, 4-H), 6.92 (1H, br s, 22-H), 7.02 (1H, br s, 24-H), 7.69 (1H, d, 5-H), 9.50 (1H, br s, pyrrole NH), 10.95 (1H, br s, Ar-O*H*).

5-(Methylacetylamino)-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylic Acid (**10**)

A23187 (1 mM) was added with stirring in pyridinic solution at 0°C of acetic anhydride (1 mM). The temperature of the mixture was allowed to rise to 20°C. After 3 hours, the pyridine was removed, the residue extracted with ether, washed with 0.1 N HCl. The organic layer was dried over $MgSO_4$. After solvent removal, the compound was purified by TLC (eluent; EtOAc - MeOH, 80:20) and then acidified carefully with 0.1 N HCl. The yield for **10** was 94%, white foam: MP 105~106°C; $[\alpha]_{578}^{25}$ +5° (*c* 0.0025, $CHCl_3$); MS *m/z* 565 (M^+), analysis correct for the hydrochloride $C_{31}H_{40}N_3O_7Cl$ (C, H, N); ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 1.78 and 1.82 (3H, d*, NCH_3COCH_3), 3.24 and 3.27 (3H, d*, NCH_3), 3.00 and 3.14 (2H, 2×d, 9-H_A and 9-H_B), 4.18 (1H, m, 10-H), 6.25 (1H, br s, 23-H), 6.93 (1H, br s, 22-H), 7.08 (1H, br s, 24-H), 7.30 (1H, d, 4-H), 7.80 (1H, d, 5-H), 10.0 (1H, br s, pyrrole NH).

5-(Methyltrifluoroacetylamino)-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylic Acid (**11**)

This compound was obtained from trifluoro acetic anhydride and A23187 by the method described previously. Yield 90%, white foam: MP 86~87°C; $[\alpha]_{578}^{25}$ +16.6° (*c* 0.0187, $CHCl_3$); MS *m/z* 619 (M^+), *Anal* $C_{31}H_{36}N_3O_7F_3$ (C, H, N); ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 3.32 and 3.39 (3H, d, NCH_3), 3.00 and 3.12 (2H, 2×d, 9-H_A and 9-H_B), 4.20 (1H, m, 10-H), 6.22 (1H, br s, 23-H), 6.91 (1H, br s, 22-H), 7.04 (1H, br s, 24-H), 7.36 (1H, d, 4-H), 7.80 (1H, d, 5-H), 9.90 (1H, br s, pyrrole NH).

* May exist as a mixture of two observable conformers with regard to the $N(CH_3)COCH_3$ group.

5-(Ethylmethylamino)-2-((3,9,11-trimethyl-8-(1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl)-1,7-dioxaspiro-(5.5)-undec-2-yl)methyl)-4-benzoxazolecarboxylic Acid (**9**)

To a solution of A23187 (200 mg) in MeOH (50 ml) was added KOH (33 mg). Ethyliodide (1 ml) was added dropwise to the stirred solution at 10°C and the mixture left overnight at room temp. After removal of the solvent, the residue was dissolved in $CHCl_3$ and filtered. The evaporation of the solution yielded **9** (120 mg), white foam: MP 95~96°C; $[\alpha]_{578}^{25}$ −68° (*c* 0.019, $CHCl_3$); *m/z* (M), found 551.2999, calcd 551.2985 for $C_{31}H_{41}N_3O_6$; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm/TMS 1.11 (3H, t, NCH_2CH_3), 3.17 (2H, q, NCH_2CH_3), 4.16 (1H, m, 10-H), 6.24 (1H, m, 23-H), 6.94 (1H, m, 22- H), 7.28 (1H, br s, 24-H), 7.36 (1H, d, 4-H), 7.75 (1H, d, 5-H), 10.85 (1H, br s, pyrrole NH). ^{13}C NMR spectra (BBD and J-Mod) confirmed structures of compounds **6**, **7**, **9**, **10**, **11**.

Calcium and Magnesium Two-phase Extractions

The experimental conditions were those of ref 22.

Decomplexation Kinetics in the Two-phase System

The dimeric complex prepared by exact neutralization of A23187 by $Mg(OH)_2$ or $Ca(OH)_2$ was dissolved in the toluene - butanol (70: 30) phase at the concentration 0.5×10^{-4} M. Five ml of this solution were poured carefully into a cell containing 5 ml of an aqueous buffered solution (tris-β,β'-dimethylglutaric acid) of variable pH. The two phases were stirred separately at 600 rot/minute without disturbing the interface. At regular periods, 1 ml of the aqueous solution was taken up with a syringe and the Ca^{++} and Mg^{++} content measured by atomic absorption, 1 ml of aqueous buffered solution was added to the cell to keep a constant aqueous volume.

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