Journal of Inclusion Phenomena **5** (1987), 99–102.

MODIFICATIONS OF BENZOXAZOLE RING SUBSTITUENTS IN A.23187 (CALCIMYCIN).EFFECT ON CATION CARRIER PROPERTIES.

M. Prudhomme,G. Dauphin,J. Guyot and G. Jeminet
Université de Clermont II,Laboratoire de Chimie Organique Biologique,U.A. 485 du CNRS,BP 45
63170 AUBIERE, FRANCE
N. Gresh
Institut de Biologie Physico-Chimique,Laboratoire de Biochimie Théorique associé au CNRS,13 rue Pierre et Marie Curie, 75005 PARIS, FRANCE

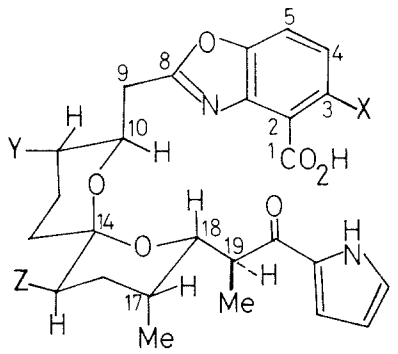
EXTENDED ABSTRACT

Introduction. Calcimycin (or A.23187) belongs to the growing family of bacterial carboxylic polyether ionophores(1).It was isolated from a strain of Streptomyces Chartreusis NRRL 3882 (2).

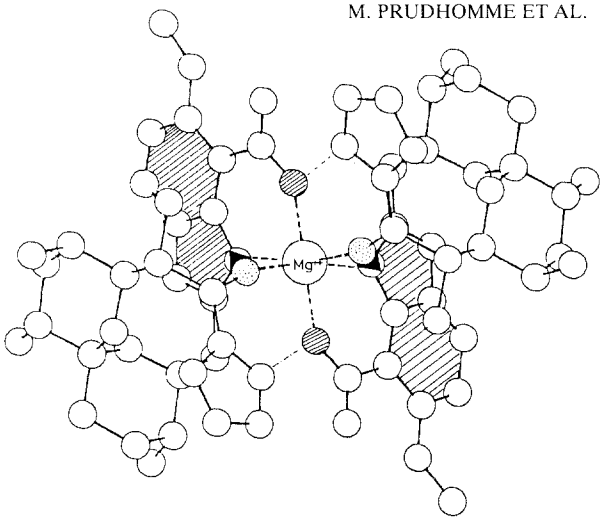
This ionophore is able to carry selectively alkaline-earth cations through membrane phases by an antiport mechanism ($M^{++} \leftrightarrows 2H^{+}$);with a transport efficiency in favour of Ca^{++} versus Mg^{++} in biological membranes(3).Owing to its specificity, calcimycin is universally used as a tool to investigate the role of the second messenger calcium in physiological processes.

However,the different steps of the transport are not well understood, not what make this molecule so efficient.Our purpose was to get information,especially as regards to the formation of the $(A.23187)_2{\cdot}M$ lipophilic complex which diffuses through the membrane and its dissociation at the interface, via a chemico-structural approach.

Solid state studies for 2:1 complexes revealed that magnesium (see below)(6) and calcium (7) coordination spheres are different.Nevertheless,in both cases the benzoxazole moiety appears to play a key role in the specific complexation of the cationic guest.In a first set of experiments we carried out chemical modifications of this part of the molecule obtained by fermentation.



Calcimycin X = $NHCH_3$ Y = Z = CH_3
microbial analogues recently isolated:
X 14885A X = OH Y = CH_3 Z = H (4)
CP 61405 X = OH Y = Z = H (5)

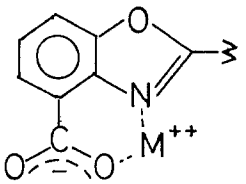


Representation of the $(A\ 23187)_2^{--}$. Mg++ complex from X-ray data (6). (▶, ◍, ⊙, coordination sites)

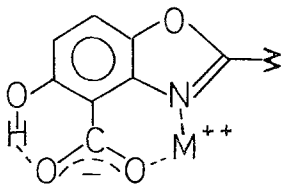
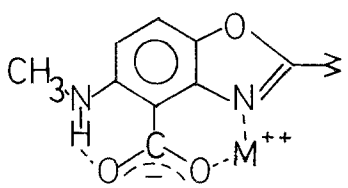
Semi-synthesis. From a selective cleavage of the oxazole ring we have worked out a semi synthetic approach in several steps which provided suitably designed analogues, closely related with respect to their overall stereochemistry, with only the benzoxazole ring substituents modified (8).

Cation carrier properties. Calcimycin has been studied in detail using the biphasic model system water/toluene-butanol, 70:30 (9); accordingly we chose this system for the sake of consistency, to get an overall characterization of the analogues with regard to their cation extraction and liberation abilities.

From the physico-chemical results, the prominent role of the 2-carboxyl benzoxazole sequence in the formation and stability of complexes was emerging.

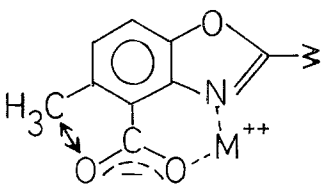
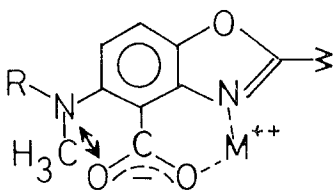


Further, electron-donating substituents H-bonded with the -COOH group brought an additional stabilizing factor for the 2:1 associations.

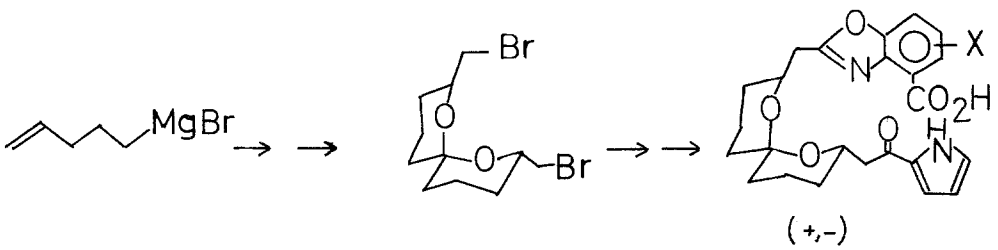
"ab initio" computations carried out independently on models confirmed that binding energies were very sensitive to the nature of the substituents(10).

Bulky substituents,hindering the carboxylic group, strongly disturbed the coordination sphere and destabilized the complexes.

A difference was observed between calcium and magnesium essentially for the initial rates of decomplexation giving in all cases $Ca^{++} \gg Mg^{++}$ (8).

Conformation. The preferential conformations of the analogues, in the acid form,were investigated by ^1H NMR(1D and 2D,300MHz); a common behaviour was noted.In $CDCl_3$,a compact globular form was postulated with a head-to-tail intramolecular chelation, similar to that one depicted for calcimycin by X-ray analysis (2).In CD_3OD,the C_8-C_9-C_{10} part was affected,corresponding to a facilited rotation of the benzoxazole ring,this conformational change could be also induced at the water/membrane interface.In both solvents,H18-H19 stayed strictly antiperiplanar suggesting a more rigid C18-C19-C20 arm.In order to examine the possible role of the 1-3 interaction between Me17 and Me19 for the preferential conformation observed,we have undertaken the synthesis of a biomimetic model with free rotating arms.Work is in progress.



In conclusion,calcimycin which is a small molecule compared to proteinic systems for instance,appears to be precisely biosynthetized for the recognition of calcium and magnesium by a specific molecular design and constitutes a model which is different from the multidentate macrocycles under investigation at the moment.

REFERENCES

1- J.W. Westly: Notation and classification.(Polyether antibiotics,naturally occuring ionophores V-1,Ed. J.W. Westly), pp. 1-20,Marcel Dekker(1982)
2- M.O. Chaney,P.V.Demarco,N.D. Jones and J.L. Occolowitz: J. Am. Chem. Soc. 96,1932(1974).
3- M. Prudhomme,J. Guyot and G. Jeminet: J. antibiotics. 39, 934(1986)
4- J.W. Westley,C.M.Liu,J.F. Blount,L.H. Sello,N. Troupe and P.A. Miller: J. antibiotics. 36,1275(1983)
5- W.D. Celmer,W.P. Cullen,H. Madea and J. Tone: U.S.Patent 4,547,523(1985)
6- M. Alléaume and Y. Barrans: Can. J. Chem 63,3482(1985)
7- G.D. Smith and W.L. Duax: J. Am. Chem. Soc 98,1578(1976)
8- M. Prudhomme,G. Dauphin and G. Jeminet: J. antibiotics. 39 922(1986)
9- D.R. Pfeiffer and H.A. Lardy: Biochem. 15,935(1976)
10- N. Gresh: Nouv. J. Chim. 10,201(1986)