# BEAUVERICIN AND DIVALENT CATIONS: CRYSTAL STRUCTURE OF THE BARIUM COMPLEX

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SUMMARY

The molecular structure of the 2:2 complex of the cyclohexadepsipeptide antibiotic beauvericin with barium picrate has been determined by X-ray crystallography. The structure serves to confirm previous observations on the bimolecular behavior of beauvericin and of the ions transported by beauvercin. The intimate involvement of the anions in the coordination of the barium also explains observations that the cation specificity of beauvericin in membrane transport depends on the species of anions present.

## INTRODUCTION

Beauvericin (1) is a cyclic hexadepsipeptide of alternating D-(N-Methyl)-phenylalanyl and L-(α hydroxy) isovaleryl residues known to be active in the membrane transport of monovalent metal cations in biological systems such as mitochondria (2,3). In previous communications we have established also the effectiveness of beauvericin with group II A cations in solvent extraction and in U-tube transport (4) for which Ba > Ca, and in liposomes and bacterial chromatophores (5) for which Ca > Ba. This crystal structure determination was undertaken in an effort to explain these observations.

## METHODS

The beauvericin was synthesized in the laboratory of Dr. Roger Roeske and is the same as used previously (4). Crystals grown from a mixture of chloroform and toluene were onthorhombic, space group $P2_12_12$ with a = 28.05(9), b = 15.79(4), c = 16.99(6) Å. This is one of five crystal forms (4) of Bv Ba $Pic_2$, and is called form B. Three of the four other crystal forms of Bv · barium picrate are obviously alternative packing forms of the present structure. Only form E shows no obvious relationship.

Data were collected on the Supper-Pace automatic diffractometer to 1.1 Å resolution, giving 2,500 non-zero independent reflections. The positions of the barium atoms were readily obtained from a Patterson synthesis. Several cycles of structure factor and Fourier calculations enabled us to recognize the entire structure. Calculations were made using Stewart's X-ray 72 system of programs, CDC-6600 version.

## RESULTS

The molecular structure thus found was $(Bv \cdot Ba \cdot Pic_3 \cdot Ba \cdot Bv)^{+}Pic^{-}$. The structure has as its center two barium ions separated by the very close distance of 4.13 Å. These two cations are bridged and held together by three picrate anions extending outward radially like a three-bladed propeller with each picrate contributing two oxygen atoms to the coordination of each barium. The two beauvericin molecules are located at each end of the propeller shaft. Each beauvericin contributes the three carbonyl oxygen atoms from the three α-isovaleryl residues. The benzene ring of the phenylalanyl residues provides the packing to enclose the anions. The complex has a non-crystallographic three-fold axis of symmetry about the line connecting the barium atoms. The fourth picrate ion is found some distance away along with two toluene molecules, none of which are well resolved. Figure 1 is a drawing from the side of the complex, and Figure 2 is a view down the non-crystallographic three-fold axis.

## DISCUSSION

Picrate ions were not included in any of the many studies of the behavior of beauvericin in membrane systems. Since the picrate ions play such an important part in the structure we have presented here, it is necessary either to nominate one or more other anions or to propose a new or at least modified complex for membrane transport. Looking at our present structure it would seem possible that the complex $(Bv \cdot M^{+} \cdot Bv)$ might be stable for monovalent cations. However, the six-fold coordination thus provided would probably be inadequate for barium and possibly calcium ions as well.

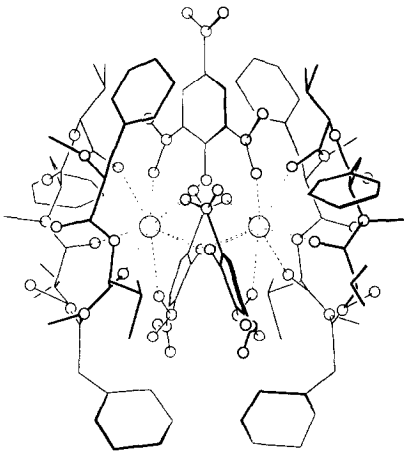

Fig. 1. The $(Bv \cdot Ba \cdot Pic_3 \cdot Ba \cdot Bv)^+$ complex as seen from the side. The imbound picrate and the two solvent molecules are not shown.

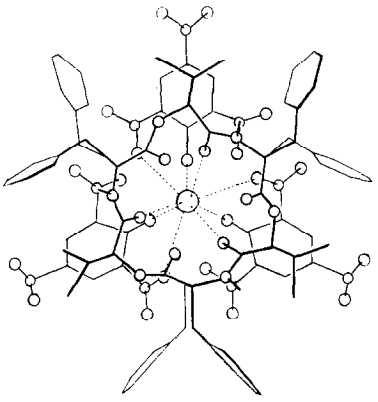

Fig. 2. The same complex viewed down the end. Only part of the distant beauvericin is given.

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In order to preserve use of the present structure it might be possible to substitute other anions for picrate. Carboxylic acids would be the most obvious choice. Indeed, the oxygen to oxygen distance in carboxylates is very nearly the same as that found in picrate. Three separate observations from membrane studies of beauvericin support this suggestion. First, Estrada-O. et al. (2) found that beauvericin in mitochondria showed different cation specificities with different carboxylic acids present. It is easy to see how the

change of anion would probably change the cation specificity of the present structure. Second, Prince, Crofts, and Steinrauf (5) found an apparent charge of plus one for calcium in the membrane transport of bacterial chromatophores in the presence of beauvericin. This could be accounted for by the transport of anions with the calcium. Third, a second-order concentration dependence on beauvericin in lipid bilayer membranes has been observed by us (6) for both mono and divalent cations, thus preserving the two beauvericin molecules per transporting unit.

It may now be possible to ask why enniatin, which has valine, leucine, or isoleucine in place of phenylalanine, does not have the divalent cation binding ability of beauvericin (5). The answer must certainly involve the benzene rings of the phenylalanyl residues which allow beauvericin to incorporate anions into the transporting complex and thus to achieve a lower net charge. The charge per surface area is probably an important consideration in the stability of complexes with polyvalent cations. In addition to binding anions the beauvericin complex reduces charge per surface area by the large area of the phenylalanyl residues.

The present structure also suggests that the N-methyl groups could well be replaced by larger groups such as isopropyl without changing the activity toward divalent cations.

The site of the potassium ion of the crystal structure of enniatin · KI was postulated (7) to be inside the cage of the six carbonyl oxygen atoms. In view of our present results this is probably incorrect since it would place the potassium too close to the carbonyl carbon atoms. When the refinement of our present structure is complete we plan to undertake calculations for the binding energy of the cation in alternative positions and also for the binding of other anions.

The present structure represents quite a different way of binding cations than that determined by us (8) previously for the dodecapeptide valinomycin in which a potassium ion was found to be entirely enclosed by the polypeptide

chain and not near the anion. None of the many studies on valinomycin has resulted in any influence of the anion on the cation specificity. In order to change the cation specificity it would presumably be necessary to alter the amino acid sequence. Thus beauvericin, with the ability to have its cation specificity altered after synthesis by the binding of different anions, represents a more advanced concept of function.

In many ways beauvericin may be considered to be an intermediate between valinomycin and alamethicin (9). Because of the second-order dependence on both cation and antibiotic (6) beauvericin will go from negligible transport to full saturation in a much smaller range of concentration than would valinomycin. Such behavior is somewhat similar to the off-on behavior of alamethicin (9). Also, beauvericin, second-order, is intermediate between valinomycin, which is first-order with respect to cation concentration, and the sixth-order behavior of alamethicin.

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