THE BINDING OF BARIUM AND CALCIUM IONS BY THE ANTIBIOTIC BEAUVERICIN

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SUMMARY

The ion-transporting antibiotic beauvericin has been shown to have a high affinity for calcium and barium ions in addition to the more usual affinity for monovalent cations. As judged by crystallization, extraction into organic solvent, and U-tube transport the cation selectivity is Rb>Ba>K>Na>>Ca>>>Li. For these studies an improved method for the synthesis of beauvericin has been developed.

INTRODUCTION

Beauvericin (Fig. 1) is a cyclic hexadepsipeptide antibiotic of the enniatin family, containing in alternating sequence three D-α-hydroxyisovaleryl and three N-methyl-L-phenylalanyl residues (1). It has been synthesized (2) and studied by ^{13}C-NMR spectra (3) as the free molecule and as the alkali ion complexes. In general, the complexing and the antimicrobial properties were found to be very similar to those of the enniatins. However, Dorschner and Lardy (4) have found that beauvericin stimulated the swelling of rat liver mitochondria less with sodium than with any other alkali ion. The same was true for the rate of hydrolysis of ATP. Estrada-O et al. (5) found a variable selectivity for alkali ions by mitochondria in the presence of beauvericin depending on the anion present.

Because of the curious behavior of beauvericin in biological systems, we believe that the molecular structure and membrane transport properties of beauvericin should be investigated in simple systems. We have carried out an improved synthesis of beauvericin to provide a source of material and to produce analogues of beauvericin for future study.

METHODS AND MATERIALS

Synthesis: Beauvericin was synthesized according to Scheme I, starting with

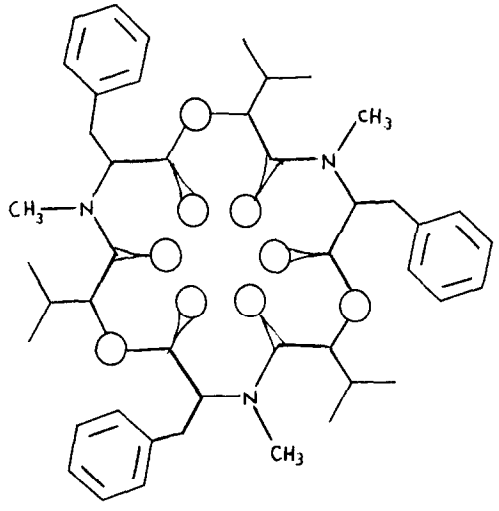
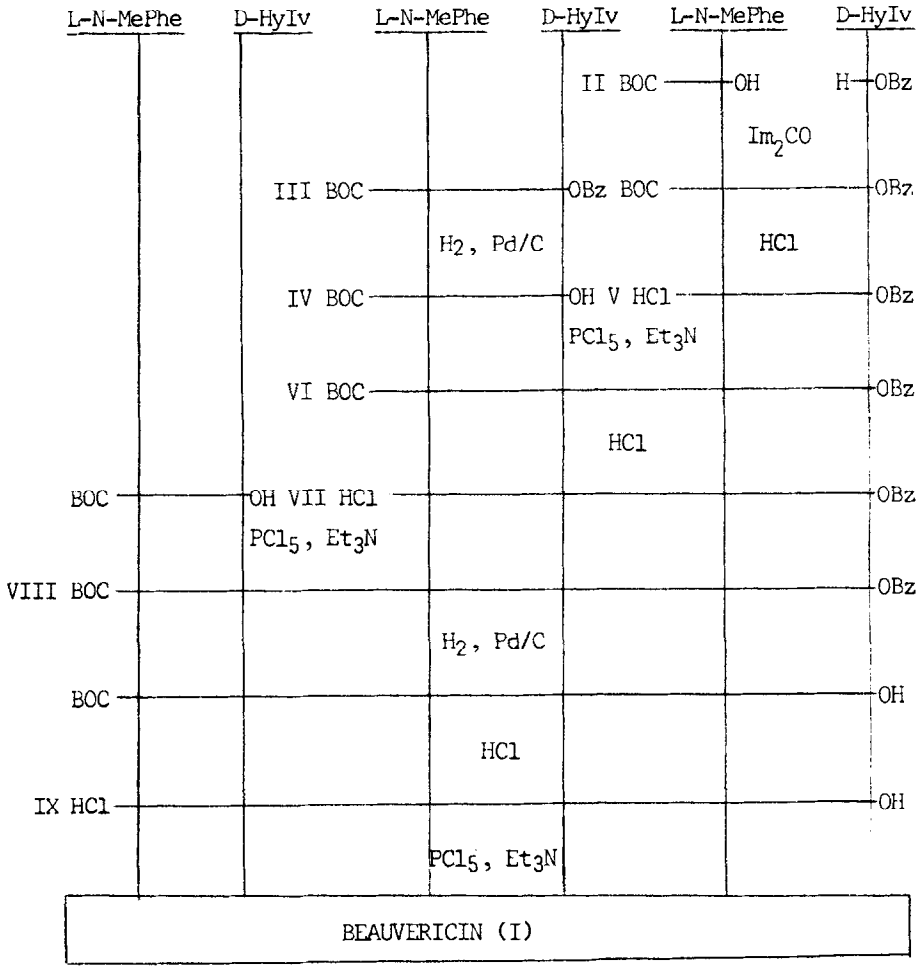


Figure 1. Beauvericin, with no attempt to represent the active conformation.

BOC-N-methyl-L-phenylalanine and benzyl-D-α-hydroxyisovalerate (6). The BOC-N-methyl-L-phenylalanine was prepared by methylation of BOC-L-phenylalanine with methyl iodide and sodium hydride (7). Carbonyldiimidazole was used to form the ester bonds and phosphorus pentachloride was used for the amide couplings. The final cyclization was carried out by the acid chloride method in 0.75 mm solution in dichloromethane to give pure beauvericin in 63% yield after one recrystallization. The NMR and mass spectra were identical to those of the natural product. The synthetic material, after recrystallization from hexane, melted at 148-149°. Yields and melting points for intermediates are given in Table I.

Extraction into Organic Solvent: Beauvericin and similar antibiotics have the ability to extract the picrate salts of metal cations from aqueous solution into organic solvents. Aqueous solutions at pH 7 were made containing 10^{-3} M picrate and 0.1 M of one of the metal chlorides given in Table II. Mixtures of 5 ml of each of these solutions were shaken at 25° C for one day with 5 ml of methylene chloride containing 2.37×10^{-4} M beauvericin. The layers were separated and the amount of picrate in each was measured by the ultraviolet absorbance. The molar extinction coefficient for picrate in water was taken to

SCHEME I



Scheme 1. An outline of the synthesis of beauvericin.
Abbreviations: BOC: tertiary butyl oxycarbonyl
-OBZ: benzyl ester
Im_2CO: carbonyl diimidazole
HyIv: D-α-hydroxyisovaleric acid

be 14,200 at 356 nm and 16,100 at 366 nm in methylene chloride. Results of several determinations for each cation are given in Table II. Extraction coefficients are calculated from

$$K_e = \frac{[\text{Beau}\cdot M\cdot P]\ \text{org}}{[M^+]\ \text{aq}\ [P^-]\ \text{aq}\ [\text{Beau}]\ \text{org}}$$

$$K_e = \frac{[\text{Beau}\cdot M\cdot P_2]\ \text{org}}{[M^{++}]\ \text{aq}\ [P^-]^2\ \text{aq}\ [\text{Beau}]\ \text{org}}$$

for the monovalent and divalent cations, respectively. However, the possibil-

TABLE I

Compound	I	II	III	IV	V	VI	VII	IX
% yield	63	60	87	94	81	76	93	73
m.p. (°C)	148-9	175-7*	oil	78-9	125-6	121-3	123-5	161-3

* as the dicyclohexylamine salt

Table I. Yields and melting points of compounds isolated in the synthesis of beauvericin as given in Scheme 1.

TABLE II

Cation	Ke	% Beauvericin complexed	Initial U-tube rate (Δ OD/day)
Li^+	4.5 (8) x 10^2	4.3 (7)	.13 (5)
Na^+	7.7 (7) x 10^2	7.1 (6)	.57 (10)
K^+	1.9 (7) x 10^3	16.0 (6)	.60 (9)
Rb^+	1.9 (9) x 10^3	16.0 (8)	.63 (10)
Ca^{++}	7.3 (3) x 10^5	6.8 (3)	.38 (10)
Ba^{++}	1.4 (7) x 10^6	12.3 (7)	.58 (12)

Table II. Association constants and percent complexation of beauvericin by metal cations and picrate, and initial rates of U-tube transport.

ity exists that the dominant species in organic solvents may be a dimer of that given above.

Crystallization of Cation Complexes: The complexes were prepared by mixing 10 mg of beauvericin dissolved in 10 ml of acetone with 10 ml of water containing sufficient metal picrate to form the 1:1 complex. The mixture was then evaporated to dryness. The calcium picrate complex was dissolved in benzene and recrystallized by the addition of N-nonane. All other complexes were dissolved

TABLE III

Metal Picrate	None	Na	K	Rb	Ca	Ba(A)	Ba(B)	Ba(C)	Ba(D)	Ba(E)
Space Group	P222	hexagonal	hexagonal	R3	?	$P2_1$	$P2_12_12$	$P2_12_12_1$	$P2_1$	$P2_1$
a	15.127	large	47.4	26.43	?	19.45	15.79	17.65	16.0	27.80
b	15.651	large	47.4	26.43	?	30.06	28.05	48.54	17.6	9.78
c	19.04	large	30.1	47.29	?	26.07	16.99	16.07	48.5	27.99
γ	90°	120°	120°	120°	?	90.0°	90°	90°	91½°	101½°
Molecules										
asymmetric unit	1	?	6 ?	2		4	1	2	4	2

Table III. Space groups and unit cell parameters for beauvericin, free and complexed with metal picrates.

in chloroform and recrystallized by the addition of benzene, toluene, m-xylene, or mesitylene.

U-tube Transport: Glass tubing, 1 cm in diameter, was bent into a U shape and filled to just above the bend with 20 ml of chloroform containing 1 mg of beauvericin. About 15 ml of an aqueous solution containing 1.0 M metal cation chloride plus 0.001 M metal cation picrate was placed in one arm and an equal volume of ion-free water in the other arm as described by Ashton and Steinrauf (8). The aqueous solutions in the arms were thus separated by the chloroform barrier made semi-permeable by the antibiotic. The rate of appearance of picrate on the initially ion-free side was determined by following the UV absorbance of the picrate ion as described in the previous section.

RESULTS AND DISCUSSION

Preparation and Crystallization. From the density of uncomplexed, crystalline beauvericin and the results from X-ray crystallography, the molecular weight was calculated to be 774 (15) as compared with 783 expected. Molecular weights were not attempted for any of the complexes since all contained solvent and would dry and turn to powder in a few minutes.

All crystal forms were examined by X-ray crystallography and excellent diffraction patterns were obtained, except for that of potassium which was poor, and that of sodium and calcium which were very poor. Results are given in Table III. It is remarkable that all crystal forms of the complexes have two or more molecules per asymmetric unit. The apparent exception, form B of barium, could also have an association through the two-fold axis. It is thus tempting to postulate that a dimer or tetramer may be the most stable form for the complexes of beauvericin.

The X-ray crystal structure determinations of the free beauvericin and of the rubidium complex are being undertaken by Dr. Alexander Geddes, University of Leeds, England. The calcium and barium complexes are being examined here.

Solvent Partition and U-tube Transport. Both systems depend on the ability of beauvericin to extract a neutral cation-anion combination from aqueous into

organic solvent. The association constants from solvent partition are given in Table II. The abilities of cations to form complexes with beauvericin follow the series Rb,K>Na>Li and Ba>Ca. The rates of cation transport with beauvericin as given in Table II were observed to be Rb>K>Ba>Na>Ca>Li. Thus, these two systems have the same relative affinities of beauvericin for cations, and do not show the lower affinity for sodium described by Dorschner and Lardy (4).

CONCLUSIONS

We have demonstrated that the antibiotic beauvericin can complex divalent as well as monovalent cations. This affinity for divalent metal cations has not been reported for enniatin. We are now undertaking an investigation of the behaviour of beauvericin and enniatin with divalent cations in lipid bilayer membrane systems and simple biological membrane systems. Such studies should also settle whether the active complex is the 1:1 or the 2:2 combination with cations. Unfortunately, there is yet no X-ray crystal structure for enniatin available for comparison, only an imcomplete attempt at the enniatin · KI complex (9).

Studies of beauvericin in biological systems may now have to be re-examined in light of the ability of the antibiotic to transport calcium as well as the monovalent cations.

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