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Crystal Structure of a Beauvericin–Barium Picrate Complex

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Abstract: The crystal structure of a complex of the cyclohexadepsipeptide antibiotic beauvericin (Bv) with barium picrate has been determined by single-crystal X-ray diffraction. The crystals are orthorhombic, space group $P2_12_12$, four antibiotic molecules per unit cell with dimensions a = 28.05 (8) Å, b = 15.79 (4) Å, and c = 16.99 (6) Å. The intensity data (2492) were collected on an automated diffractometer. The structure, determined by the heavy-atom method, was refined by difference Fourier and least-squares methods. The final R was 0.15 for all observed data. The structure revealed that the molecular unit was $(BvBaPic_3BaBv)^+Pic^-$, which agreed with previous observations on the behavior of the ion transport induced by beauvericin in membrane systems. The intimate involvement of the picrate anions in the coordination of the barium ion also explains observations that the cation specificity of beauvericin in membrane transport depends on the species of anions present.

Beauvericin (Bv) is a cyclic hexadepsipeptide of alternating L-*N*-methylphenylalanyl and D-α-hydroxyisovaleryl residues.[1] It belongs to the enniatin family of antibiotics which are active against Gram-positive bacteria and mycobacteria. The general formula for the family is $(\text{L-MeX-D-Hylv})_3$ where X is Ile for enniatin A, Val for enniatin B, Leu for enniatin C, and Phe for beauvericin.

Beauvericin is capable of complexing ions and transporting them through membranes in biological systems such as mitochondria.[2,3] It is capable of complexing both alkaline-earth metals and alkali-metal ions.[4,6] Although beauvericin does not have the specific cation selectivity of other antibiotics, e.g., valinomycin, it is most unusual in showing an anion-dependent cation specificity.[2] Ivanov et al.[7] have proposed enniatin complexes with 2:1 and 3:2 macrocycle:cation ratios. As mentioned in a short communication by us,[8] the crystal structure of a beauvericin–barium picrate complex shows the existence of $(BvBaPic_3BaBv)^+Pic^-$ as the structural unit. Preliminary results on the crystal structure of beauvericin–potassium picrate (our laboratory) and beauvericin–rubidium picrate (University of Leeds, England) confirm this molecular clustering as a stable and commonly occurring structural unit for beauvericin ion complexes.

The X-ray crystal structure of uncomplexed beauvericin has recently been determined[9] and also a different crystal form of the barium picrate complex by Dr. Alexander Geddes, University of Leeds (personal communication), which will allow comparison of the complexed and uncomplexed conformations with a view to explaining the mechanism of membrane transport.

Experimental Section

Crystallization and Crystal Data for Beauvericin–Barium Picrate. The beauvericin was synthesized in the laboratory of Dr. Roger Roeske and is the same as used previously.[4] Crystals of the barium picrate complex were prepared by mixing equimolar amounts of beauvericin and barium picrate and crystallizing by slow evaporation from a chloroform–toluene mixture. The crystal system is orthorhombic, space group $P2_12_12$, with unit cell dimensions a = 28.05 (9) Å, b = 15.79 (4) Å, c = 16.99 (6) Å, V = 7525 Å^3, Z = 4. This is one of five known crystal forms of beauvericin–barium picrate and is called form B.[4] Three of the four other crystal forms of the complex are obviously alternative packing arrangements of the present structure. Only one form, E, shows no obvious relationship.[4]

The cell parameters were determined from calibrated Weissenberg films using Cu Kα radiation. The observed density using flotation in $ZnCl_2$-water was 1.40 (4) g/cm^3. The calculated density with one barium atom, one beauvericin molecule, and two picrate anions in the crystallographic asymmetric unit was considerably less (1.21 g/cm^3) suggesting the presence of solvent molecules. The calculated density, including the two solvent molecules in the asymmetric unit as determined from the structure analysis, was 1.37 g/cm^3.

Intensities were measured on a Supper-Pace automated diffractometer which uses Weissenberg geometry. Two independent sets of data on separate crystals (0.2 × 0.2 × 0.8 and 0.2 × 0.2 × 0.4 mm) were collected about the a and c axes using a scanning method described previously.[5] Mo Kα radiation was used to minimize absorption correction; no absorption corrections were applied. The maximum error in the standard reflections due to radiation damage was 5% in intensity. All reflections with intensities greater than one standard deviation were used. The two data sets were scaled together and

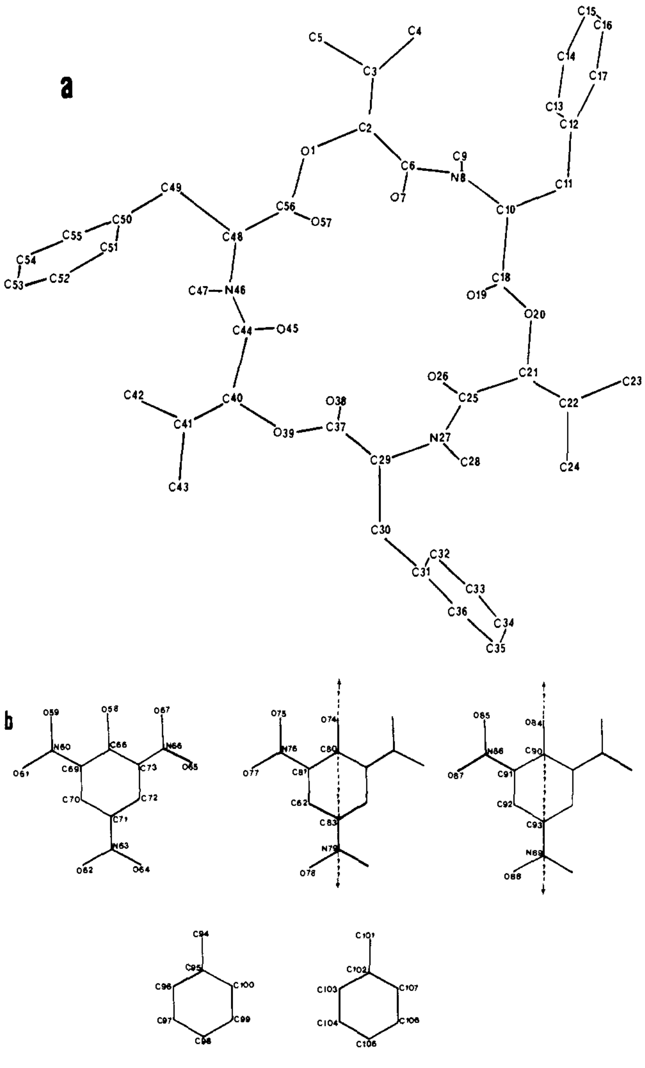

Figure 1. Numbering scheme of (a) the beauvericin molecule and (b) the picrate and toluene molecules in the crystallographic asymmetric unit. Two picrate molecules lie in special positions with one-half of the molecule in the asymmetric unit. The crystal twofold axis which generates the other half is shown by a dotted arrow.

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Table I. Fractional Atomic Parameters and Thermal Parameters with Their Estimated Standard Deviations Where Applicable[a]

atom no.	x	y	z	U
Ba	1639(10)	12748(22)	−18991(18)	*
O(1)	−84(13)	3050(23)	−3843(20)	5.1(11)
C(2)	396(23)	3009(45)	−4232(40)	7.1(22)
C(3)	392(27)	2386(52)	−4888(49)	9.6(26)
C(4)	−67(29)	2634(51)	−5416(44)	11.3(27)
C(5)	789(35)	2590(66)	−5280(60)	16.7(38)
C(6)	709(18)	2675(35)	−3609(29)	2.6(14)
O(7)	540(11)	2039(22)	−3227(21)	3.8(10)
N(8)	1152(14)	2826(27)	−3492(23)	3.1(12)
C(9)	1293(17)	3698(38)	−3781(28)	4.0(14)
C(10)	1456(14)	2343(28)	−2937(25)	2.0(12)
C(11)	1952(0)	2259(0)	−3318(0)	1.8(13)
C(12)	1967(0)	1853(0)	−4141(0)	3.9(16)
C(13)	1732(0)	1084(0)	−4280(0)	7.7(23)
C(14)	1746(0)	714(0)	−5028(0)	8.8(26)
C(15)	1994(0)	1115(0)	−5639(0)	12.9(31)
C(16)	2227(0)	1885(0)	−5502(0)	9.2(26)
C(17)	2214(0)	2253(0)	−4753(0)	6.6(20)
C(18)	1460(16)	2765(32)	−2071(28)	2.8(14)
O(19)	1329(13)	3463(24)	−1956(25)	7.0(12)
O(20)	1618(11)	2155(21)	−1636(18)	3.2(9)
C(21)	1605(20)	2353(38)	−725(35)	5.3(18)
C(22)	1815(18)	1577(35)	−493(30)	4.4(16)
C(23)	2369(18)	1543(35)	−753(31)	4.4(16)
C(24)	1881(25)	1848(49)	461(44)	9.9(25)
C(25)	1099(30)	2499(57)	−5544(53)	12.9(32)
O(26)	800(11)	1970(22)	−850(19)	3.5(9)
N(27)	961(15)	2907(28)	−33(25)	3.7(13)
C(28)	1225(19)	3688(43)	329(32)	5.3(17)
C(29)	397(17)	2917(33)	231(29)	2.6(15)
C(30)	409(0)	3061(0)	1178(0)	1.0(12)
C(31)	678(0)	2420(0)	1700(0)	6.8(21)
C(32)	590(0)	1552(0)	1620(0)	6.8(23)
C(33)	835(0)	971(0)	2093(0)	7.6(23)
C(34)	1166(0)	1258(0)	2646(0)	14.2(35)
C(35)	1255(0)	2127(0)	2727(0)	8.3(26)
C(36)	1010(0)	2708(0)	2253(0)	4.3(17)
C(37)	158(25)	3427(45)	−281(39)	6.3(22)
C(38)	273(16)	3938(31)	−631(26)	1.2(13)
O(39)	−311(12)	3194(23)	−139(21)	5.2(11)
C(40)	−685(21)	3629(45)	−666(35)	6.1(19)
C(41)	−1144(26)	3153(49)	−369(45)	10.2(25)
C(42)	−1194(30)	3415(57)	525(52)	13.4(32)
C(43)	−1540(23)	3495(44)	−777(39)	7.2(22)
C(44)	−602(14)	3192(33)	−1541(24)	2.0(12)
O(45)	−416(12)	2533(25)	−1546(20)	3.9(11)
N(46)	−679(10)	3788(24)	−2070(19)	1.5(13)
C(47)	−745(16)	4759(28)	−2089(26)	1.4(9)
C(48)	−635(17)	3448(34)	−2780(29)	3.2(16)
C(49)	−1086(0)	3638(0)	−3453(0)	6.2(18)
C(50)	−1572(0)	3484(0)	−3054(0)	9.6(24)
C(51)	−1871(0)	4169(0)	−2882(0)	4.2(17)
C(52)	−2312(0)	4029(0)	−2519(0)	9.2(26)
C(53)	−2455(0)	3205(0)	−2328(0)	6.7(19)
C(54)	−2155(0)	2519(0)	−2502(0)	6.3(20)
C(55)	−1714(0)	2659(0)	−2864(0)	2.2(14)
C(56)	−144(24)	3488(40)	−3049(38)	7.0(19)
C(57)	152(17)	3853(29)	−2900(23)	1.3(9)
O(58)	−563(0)	201(0)	−1404(0)	1.5(8)
O(59)	−1684(15)	263(28)	−2683(24)	8.1(14)
N(60)	−1448(0)	−47(0)	−2231(0)	3.2(12)
O(61)	−1058(15)	−465(29)	−2440(26)	8.0(15)
O(62)	−2624(19)	983(35)	−367(32)	12.5(19)
N(63)	−2263(0)	1087(0)	35(0)	8.1(18)
O(64)	−2270(22)	1221(46)	644(37)	15.4(23)
O(65)	−568(19)	923(35)	773(33)	11.5(20)
N(66)	−549(0)	902(0)	160(0)	13.2(29)
O(67)	−162(15)	1022(26)	−191(23)	8.1(13)
C(68)	−944(0)	399(0)	−1082(0)	6.7(19)
C(69)	−1410(0)	322(0)	−1447(0)	2.2(10)
C(70)	−1834(0)	521(0)	−1089(0)	5.8(19)
C(71)	−1822(0)	857(0)	−338(0)	6.8(20)
C(72)	−1400(0)	979(0)	64(0)	2.7(14)
C(73)	−982(0)	740(0)	−291(0)	4.9(17)
O(74)	0(0)	0(0)	−2965(25)	2.7(12)
O(75)	−617(11)	1283(24)	−3089(22)	4.7(10)
N(76)	−668(16)	958(29)	−3773(26)	4.3(13)
O(77)	−1026(17)	1278(37)	−4115(29)	9.9(16)
O(78)	−336(27)	505(50)	−6560(46)	21.1(34)
N(79)	0(0)	0(0)	−6238(47)	8.4(28)
C(80)	0(0)	0(0)	−3729(32)	2.0(17)
C(81)	−351(17)	432(35)	−4165(32)	4.1(17)
C(82)	−388(19)	462(37)	−4903(34)	5.5(18)
C(83)	0(0)	0(0)	−5268(39)	2.6(10)
O(84)	5000(0)	0(0)	−5257(0)	10.2(10)
O(85)	3937(0)	1062(0)	−3966(0)	13.8(12)
N(86)	4179(0)	581(0)	−4357(0)	15.3(12)
O(87)	4073(0)	348(0)	−5071(0)	8.6(10)
O(88)	4660(0)	299(0)	−1378(0)	8.6(10)
N(89)	5000(0)	0(0)	−1748(0)	9.5(10)
C(90)	5000(0)	0(0)	−4470(0)	13.9(11)
C(91)	4604(0)	262(0)	−3950(0)	15.0(14)
C(92)	4604(0)	279(0)	−3081(0)	14.5(13)
C(93)	5000(0)	0(0)	−2657(0)	15.6(14)
C(94)	4191(0)	2578(0)	−2604(0)	17.4(43)
C(95)	3766(0)	3050(0)	−2975(0)	14.9(36)
C(96)	3721(0)	3091(0)	−3794(0)	15.6(37)
C(97)	3335(0)	3519(0)	−4131(0)	12.6(33)
C(98)	2994(0)	3906(0)	−3648(0)	18.4(46)
C(99)	3039(0)	3864(0)	−2828(0)	15.1(37)
C(100)	3426(0)	3436(0)	−2492(0)	9.8(27)
C(101)	2798(0)	−644(0)	−4257(0)	19.5(54)
C(102)	2934(0)	−175(0)	−3494(0)	4.6(18)
C(103)	3024(0)	697(0)	−3511(0)	11.9(32)
C(104)	3147(0)	1123(0)	−2817(0)	21.7(54)
C(105)	3180(0)	676(0)	−2107(0)	27.4(77)
C(106)	3090(0)	−196(0)	−2090(0)	11.7(30)
C(107)	2967(0)	−621(0)	−2784(0)	12.4(36)

*	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Ba	3.02(16)	3.61(19)	3.51(17)	−42(22)	3(1)	−8(2)

[a] No standard deviations are given for atoms refined as a rigid body. Positional parameters have been multiplied by 10^4 except for those of the barium, which are multiplied by 10^5, and thermal parameters by 10^2. The isotropic temperature factor is of the form $T = \exp[-8\pi^2 U \sin^2\theta/\lambda^2]$ and the anisotropic temperature factor $T = \exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})]$.

merged. The average error in the structure amplitudes ($|F|$) with approximately 1100 reflections common to the two data sets was 9%. A final data set with 2492 unique reflections extending to 1.1-Å resolution was obtained. This constitutes approximately 86% of the possible reflections in the 1.1-Å sphere of resolution.

Structure Determination and Refinement. The crystal structure was solved by the heavy-atom method, the barium position being easily determined from a Patterson map. A first Fourier map calculated using the barium phases showed a false mirror plane across the X axis due to the fact that the X coordinate of the barium atom is almost 0.

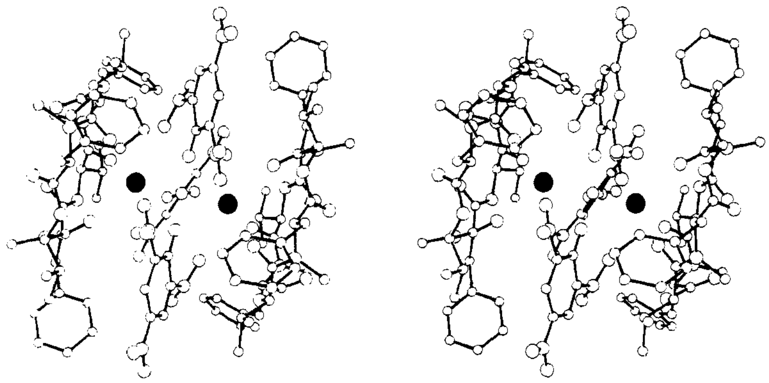

Figure 2. Stereoview of the beauvericin-barium picrate complex, viewed down the crystallographic twofold axis.

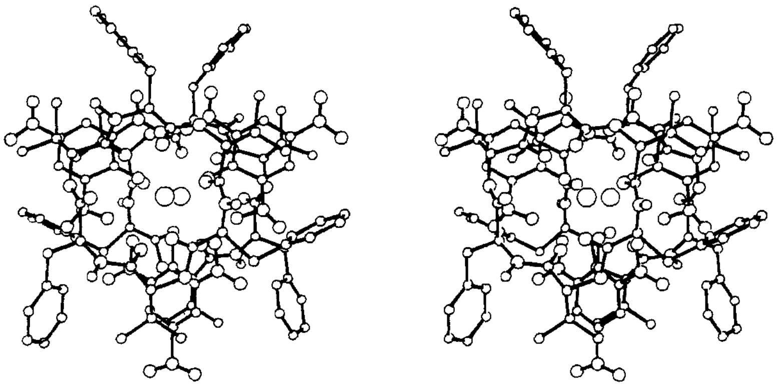

Figure 3. Stereoview of the beauvericin-barium picrate complex, viewed down the noncrystallographic threefold axis.

Table II. Intramolecular Bond Lengths (A) and Standard Deviations[a]

Beauvericin Molecule		C(40)-C(41)	1.57(10)
O(1)-C(2)	1.50(7)	C(40)-C(44)	1.66(8)
C(2)-C(3)	1.49(11·)	C(41)-C(42)	1.58(12)
C(2)-C(6)	1.47(8)	C(41)-C(43)	1.42(10)
C(3)-C(4)	1.62(11)	C(44)-O(45)	1.17(6)
C(3)-C(5)	1.34(13)	C(44)-N(46)	1.32(6)
C(6)-O(7)	1.29(6)	N(46)-C(47)	1.55(6)
C(6)-N(8)	1.28(6)	N(46)-C(48)	1.33(6)
N(8)-C(9)	1.52(7)	C(48)-C(49)	1.73(0)
N(8)-C(10)	1.48(6)	C(48)-C(56)	1.45(8)
C(10)-C(11)	1.54(0)	C(56)-O(57)	1.04(8)
C(10)-C(18)	1.61(6)	C(56)-O(1)	1.53(7)
C(18)-O(19)	1.18(6)		
C(18)-O(20)	1.29(6)	Picrate Anion in Special Position	
O(20)-C(21)	1.58(7)	N(76)-O(75)	1.28
C(21)-C(25)	1.46(8)	N(76)-O(77)	1.27
C(21)-C(22)	1.42(8)	N(79)-O(78)	1.35
C(22)-C(23)	1.62(7)	N(86)-O(85)	1.22
C(22)-C(24)	1.69(9)	N(86)-O(87)	1.30
C(25)-O(26)	1.26(6)	N(89)-O(88)	1.24
C(25)-N(27)	1.20(6)		
N(27)-C(28)	1.57(8)	Picrate Anion in General Position	
N(27)-C(29)	1.64(6)	N(60)-O(59)	1.13
C(29)-C(30)	1.63(0)	N(60)-O(61)	1.32
C(29)-C(37)	1.36(8)	N(63)-O(62)	1.23
C(37)-O(38)	1.05(8)	N(63)-O(64)	1.06
C(37)-O(39)	1.39(8)	N(66)-O(67)	1.25
O(39)-C(40)	1.54(7)	N(66)-O(65)	1.05

[a] The bond lengths and angles of the atoms involved in rigid groups are not listed (see also the section on the structure refinement).

Elucidation of the positions of the oxygen atoms coordinating to the barium allowed weighting of one of the mirror images. Subsequent cycles of structure-factor calculation and Fourier and difference Fourier synthesis yielded the positions of all of the atoms in the beauvericin molecule and the picrate anions. Two partially occupied solvent molecules (toluene) were also revealed. The limiting resolution of the data did not allow good refinement by the standard individual atom least-squares method. We therefore used the rigid-body refinement method to reduce the number of variable parameters in the refinement. The rigid groups for which the idealized parameters were used from the literature[20] include the phenyl rings of the beauvericin molecules, the picrate anions excluding the nitro groups, and the toluene solvent molecules.

With the exception of barium[11] the scattering factors were from International Tables for X-ray Crystallography.[10] In the picrate molecules the O^- form factors were used for the ionized oxygen. All computer programs except for those used in the preliminary data collection and processing (written by us) were from the X-RAY 76 system.[12] Isotropic temperature parameters were used for all atoms except barium, which was allowed to vibrate anisotropically. The final *R* value for the 2492 observed nonzero reflections was 0.15.

Results and Discussion

The numbering scheme for the beauvericin molecular complex is given in Figure 1. The stereoview of the beauvericin picrate complex viewed down the twofold and threefold axes of the complex are shown in Figures 2 and 3, respectively. The final fractional coordinates and thermal parameters are listed in Table I. The bond lengths for the structural asymmetric unit are listed in Table II and the corresponding angles in Table III. The torsional angles are listed in Table IV.

Description of the Structure. The beauvericin-barium picrate forms a complex which is essentially a dimer resulting from the action of the twofold crystallographic symmetry axis on the beauvericin, Ba^{2+}, and picrate anions of the asymmetric unit. In addition there is a noncrystallographic threefold symmetry axis about the line joining the barium ions. This pseudo-threefold axis is perpendicular to the crystal *c* axis and 12° from the *b* axis. Therefore the complex has 32 point

Table III. Bond Angles (deg) with Standard Deviations Where Applicable

Beauvericin Molecule	
C(56)-O(1)-C(2)	121(4)
O(1)-C(2)-C(3)	111(5)
O(1)-C(2)-C(6)	104(5)
C(3)-C(2)-C(6)	108(6)
C(2)-C(3)-C(5)	102(7)
C(2)-C(3)-C(4)	105(6)
C(4)-C(3)-C(5)	109(7)
C(2)-C(6)-O(7)	115(5)
C(2)-C(6)-N(8)	129(5)
O(7)-C(6)-N(8)	115(5)
C(6)-N(8)-C(9)	112(4)
C(6)-N(8)-C(10)	124(4)
C(9)-N(8)-C(10)	122(4)
N(8)-C(10)-C(11)	107(0)
N(8)-C(10)-C(18)	112(4)
C(11)-C(10)-C(18)	114(0)
C(10)-C(18)-O(19)	122(4)
C(10)-C(18)-O(20)	102(4)
O(19)-C(18)-O(20)	135(5)
C(18)-O(20)-C(21)	114(4)
O(20)-C(21)-C(22)	95(4)
O(20)-C(21)-C(25)	103(5)
C(22)-C(21)-C(25)	119(6)
C(21)-C(22)-C(23)	111(5)
C(21)-C(22)-C(24)	95(5)
C(23)-C(22)-C(24)	100(4)
C(21)-C(25)-O(26)	119(5)
C(21)-C(25)-N(27)	122(5)
O(26)-C(25)-N(27)	115(5)
C(25)-N(27)-C(28)	125(4)
C(25)-N(27)-C(29)	122(4)
C(28)-N(27)-C(29)	110(4)
N(27)-C(29)-C(30)	105(0)
N(27)-C(29)-C(37)	108(5)
C(30)-C(29)-C(37)	124(0)
C(29)-C(37)-O(38)	132(7)
C(29)-C(37)-O(39)	102(5)
O(38)-C(37)-O(39)	126(7)
C(37)-O(39)-C(40)	115(4)
O(39)-C(40)-C(41)	99(5)
O(39)-C(40)-C(44)	104(4)
C(41)-C(40)-C(44)	102(5)
C(40)-C(41)-C(42)	105(6)
C(40)-C(41)-C(43)	108(6)
C(42)-C(41)-C(43)	108(6)
C(40)-C(44)-O(45)	116(4)
C(40)-C(44)-N(46)	107(4)
O(45)-C(44)-N(46)	135(4)
C(44)-N(46)-C(47)	138(4)
C(44)-N(46)-C(48)	109(4)
C(47)-N(46)-C(48)	113(4)
N(46)-C(48)-C(49)	118(0)
N(46)-C(48)-C(56)	111(4)
C(49)-C(48)-C(56)	119(0)
C(48)-C(56)-O(57)	135(6)
O(1)-C(56)-C(48)	111(5)
O(1)-C(56)-O(57)	112(6)

Picrate Anion in General Position	
O(59)-N(60)-C(69)	120
O(59)-N(60)-O(61)	121
C(69)-N(60)-O(61)	113
O(62)-N(63)-C(71)	115
O(64)-N(63)-C(71)	120
O(62)-N(63)-O(64)	124
O(67)-N(66)-C(73)	120
O(65)-N(66)-C(73)	119
O(65)-N(66)-O(67)	121

Picrate Anion in Special Position	
O(75)-N(76)-C(81)	127
O(77)-N(76)-C(81)	122
O(75)-N(76)-O(77)	110
O(78)-N(79)-C(83)	114
O(78)-N(79)-O(78)[a]	132
O(85)-N(86)-O(87)	124
O(85)-N(86)-C(91)	114
O(87)-N(86)-C(91)	122
O(88)-N(89)-C(93)	121
O(88)-N(89)-O(88)[a]	119

[a] These atoms are obtained using the equivalent position $-x$, $-y$, z.

symmetry. The fourth picrate ion, necessary for electrical neutrality, is situated on a crystallographic twofold axis in another part of the cell. The asymmetric unit also contains two toluene molecules which are not well resolved and are not intimately connected to the complex.

The structure therefore is a modification of the "sandwich" complex proposed by Ovchinnikov[13] with the important addition of three picrate molecules involved in the coordination to the barium ions. Coordination to each barium ion is provided by the three amide group oxygens from beauvericin and the three phenolic and three nitro group oxygens (considerably weaker bonds) from the picrates. The coordination distances are given in Table V. The compactness of this complex is shown by the short $Ba^{2+} \cdots Ba^{2+}$ distance of 4.132 (4) Å. There is an overall remarkable similarity of this coordination with that shown for the "double binding" Ca^{2+} site in thermolysin,[14] where the $Ca^{2+} \cdots Ca^{2+}$ distance is 3.8 Å and the six coordinating ligands to Ca^{2+} come from two water molecules, an asparaginyl residue, an aspartyl, and two glutamyl residues. The aspartyl and two glutamyl residues form oxygen bridges between the two Ca^{2+}, one oxygen of the carboxylate group coordinating to $Ca_1{}^{2+}$ of the pair and the other to $Ca_2{}^{2+}$ of the pair. The remaining ligands to Ca^{2+} come from water, an isoleucyl, and another aspartyl residue. Thus, there are three bridging carboxylate groups. In the beauvericin-barium picrate complex there is a similar bridging between the two barium ions by means of the phenolate oxygen of the picrate anions and six strong ligands to each barium ion. In our opinion calcium could substitute for barium to give an isostructural complex with beauvericin and picrate.

The beauvericin-picrate complex is highly hydrophobic on the surface owing to the presence of the phenyl, isopropyl, and *N*-methyl groups of the beauvericin, the phenyl and the isopropyl groups providing a shielding for the "sandwiched" ions (Figure 2). The picrates are arranged as a three-bladed "propeller" around the noncrystallographic threefold axis of the complex (Figure 3). The beauvericin molecule is in the *P* conformation[13] since it does not deviate significantly from absolute threefold symmetry. This is evident from the conformation angles (Table IV). It is interesting to note that the phenyl and isopropyl side chains also follow closely the noncrystallographic threefold symmetry of the complex. In the crystal structure of uncomplexed beauvericin,[9] the side chains do not follow the threefold symmetry of the molecule.

The beauvericin molecule is disk shaped with the amide carbonyl groups projecting to one side of the molecule and the ester carbonyl and amide *N*-methyl groups projecting to the other side. The beauvericin dimers in this crystal form stack head to tail on top of one another creating almost continuous columns of approximately coaxial cylindrical density

Table IV. Beauvericin Torsional Angles (deg)[a]

3_1		3_2		3_3	
C(56)-O(1)-C(2)-C(3)	166	C(18)-O(20)-C(21)-C(22)	182	C(37)-O(39)-C(40)-C(41)	176
C(56)-O(1)-C(2)-C(6)	51	C(18)-O(20)-C(21)-C(25)	61	C(37)-O(39)-C(40)-C(44)	71
O(1)-C(2)-C(3)-C(5)	165	O(20)-C(21)-C(22)-C(24)	172	O(39)-C(40)-C(41)-C(43)	178
O(1)-C(2)-C(3)-C(4)	51	O(20)-C(21)-C(22)-C(23)	69	O(39)-O(40)-C(41)-C(42)	64
O(1)-C(2)-C(6)-O(7)	46	O(20)-C(21)-C(25)-O(26)	47	O(39)-C(40)-C(44)-C(45)	22
O(1)-C(2)-C(6)-N(8)	−148	O(20)-C(21)-C(25)-N(27)	−155	O(39)-C(40)-C(44)-C(46)	−144
C(2)-C(6)-N(8)-C(9)	29	O(21)-C(25)-N(27)-C(28)	32	O(40)-C(44)-N(46)-C(47)	12
C(2)-C(6)-N(8)-C(10)	−169	C(21)-C(25)-N(27)-C(29)	−169	C(40)-C(44)-N(46)-C(48)	−177
C(6)-N(8)-C(10)-C(11)	143	C(25)-N(27)-C(29)-C(30)	148	C(44)-N(46)-C(48)-C(49)	131
C(6)-N(8)-C(10)-C(18)	−91	C(25)-N(27)-C(29)-C(37)	−78	C(44)-N(46)-C(48)-C(56)	−82
N(8)-C(10)-C(11)-C(12)	−56	N(27)-C(29)-C(30)-C(31)	−58	N(46)-C(48)-C(49)-C(50)	−47
C(10)-C(11)-C(12)-C(13)	−50	C(29)-C(30)-C(31)-C(32)	−51	C(48)-C(49)-C(50)-C(55)	−71
C(10)-C(11)-C(12)-C(17)	130	C(29)-C(30)-C(31)-C(36)	130	C(48)-C(49)-C(50)-C(51)	109
N(8)-C(10)-C(18)-O(19)	−16	N(27)-C(29)-C(37)-O(38)	−26	N(46)-C(48)-C(56)-C(57)	−21
N(8)-C(10)-C(18)-O(20)	162	N(27)-C(29)-C(37)-O(39)	164	N(46)-C(48)-C(56)-O(1)	176
C(10)-C(18)-O(20)-C(21)	−173	C(29)-C(37)-O(39)-C(40)	−177	C(48)-C(56)-O(1)-C(2)	−180
		Picrate Anion (General Position)			
C(70)-C(69)-N(60)-O(61)	200	C(68)-C(73)-N(66)-O(65)	203	C(70)-C(71)-N(63)-O(64)	167
C(70)-C(69)-N(60)-O(59)	48	C(68)-C(73)-N(66)-O(67)	24	C(70)-C(71)-N(63)-O(62)	−2
C(68)-C(69)-N(60)-O(61)	16	C(72)-C(73)-N(66)-O(65)	27	C(72)-C(71)-N(63)-O(64)	−13
C(68)-C(69)-N(60)-O(59)	−136	C(72)-C(73)-N(66)-O(67)	−152	C(72)-C(71)-N(63)-O(62)	178
		Picrate Anions (Special Position)			
C(80)-C(81)-N(76)-O(75)	20	C(90)-C(91)-N(86)-O(87)	27		
C(80)-C(81)-N(76)-O(77)	−171	C(90)-C(91)-N(86)-O(85)	−155		
C(82)-C(81)-N(76)-O(75)	−154	C(92)-C(91)-N(86)-C(87)	−157		
C(82)-C(81)-N(76)-O(77)	15	C(92)-C(91)-N(86)-O(85)	22		
C(82)-C(83)-N(79)-O(78)	0	C(92)-C(93)-N(89)-O(88)	0		

[a] Average estimated standard deviation for the torsional angles is 6°. The notation 3_1, 3_2, 3_3 refers to the three units related by the noncrystallographic threefold symmetry.

Table V. Coordination Distances (Å) around the Barium Ion

coordinating atoms	distance	coordinating atoms	distance
Ba···O(7)	2.77(4)	Ba···O(58)[a]	2.72(1)
Ba···O(26)	2.75(3)	Ba···O(67)	3.07(4)
Ba···O(45)	2.64(4)	Ba···O(75)	2.98(3)
Ba···O(58)	2.78(1)	Ba···O(61)[a]	2.96(4)
Ba···O(74)	2.75(3)		

[a] These atoms are obtained using the equivalent position $-x$, $-y$, z.

Table VI. Comparison of the Twist of the Nitro Groups with Respect to the Benzene Ring in the Picrate Anion for Several Crystalline Complexes[a]

	twist of the nitro groups, deg			
complex	ortho	ortho	para	ref
potassium picrate	25.7	25.7	−2.2	20
ammonium picrate	26.7	26.7	−5.0	20
thallium picrate	39.4	1.9	15.0	21
polyether dicarboxylic acid potassium picrate	55.8	−2.4	7.2	23
serotonin picrate	36.4	2.8	−2.1	22
prolinomycin rubidium picrate	60.3	−16.6	2.7	26
valinomycin potassium picrate	41.7	34.9	−0.3	25
beauvericin barium picrate				
(general position)	32.2	25.3	−7.5	present
(special position 1)	17.5	17.5	0.0	present
(special position 2)	24.3	24.3	0.0	present

[a] The twist is calculated by averaging for each nitro group the four dihedral angles with respect to the benzene ring.

throughout the whole crystal. There is no interaction between parallel cylindrical columns, the space between being occupied by the extra picrate anions required for charge neutrality and the solvent molecules. It is of interest to compare the conformation of the picrate molecules in the beauvericin complex with the crystal structures of picrate complexed with potassium,[20] ammonium,[20] thallium,[21] serotonin,[22] polyether dicarboxylic acid,[23] rubidium prolinomycin,[24] and potassium valinomycin.[25] In all these structures, the conformation of the *p*-nitro group of the picrate anion is significantly different from that of the *o*-nitro groups. While the *p*-nitro groups are close to being in the plane of the benzene ring, the *o*-nitro groups are twisted significantly from this plane, by a maximum of 60° (Table VI). This generalization appears to hold in the case of the present structure also; the average twists of the *p*- and *o*-nitro groups of the picrate molecules are −3 and 24°, respectively. The significant twist of the *o*-nitro groups compared to the *p*-nitro group of the picrate anion under very different environments suggests that this in an *intra*molecular effect, possibly due to overcrowding of the nitro groups and the phenolic oxygen.

The structure of form D of beauvericin-barium picrate has been solved by Dr. Geddes at the University of Leeds (personal communication). Form D is related to the present structure by a doubling of the *a* axis with $P2_1$ as the space group. Form D has two clusters of (BvBaPic$_3$BaBv)$^+$Pic$^-$ in the asymmetric unit which differ most significantly from the cluster described here by the orientation of the benzene rings of the phenylanalyl residues. The coordinations of the anions and cations of the two crystal forms are the same to within experimental error. The beauvericin dimers form continuous columns in the crystal of form D also. Less solvent was found in form D, two molecules of benzene per four molecules of beauvericin vs. two molecules of toluene per one molecule of beauvericin found in this work. The perpendicular distances involving the barium (Ba) atom and the centers of the beauvericin (Bv) molecules are similar; our values followed in parentheses by Leed's values are Ba to Ba 4.13 (4.18) Å, Ba to nearest Bv 2.77 (2.79) Å, and Bv to Bv within a complex 9.67 (9.76) Å. The center of the beauvericin molecule is calculated by averaging the midpoints of the lines joining the carbonyl oxygen atoms related by the approximate molecular center of symmetry of the beauvericin molecules.

Structure and Membrane Function Relationship. The intimate involvement of the anion in the beauvericin complex is a feature which has not previously been observed for ionophore-ion complexes and the presence of anion in the ion-transporting species in solution agrees with the experimental results using artificial membranes described below. Anions have previously been observed in the coordination scheme of some of the cyclic polyether-cation complexes;[17,18] however, involvement of the anion in the transporting mechanism has not been observed for these ionophores. Tosteson and coworkers[19] report some effect of anion in transport studies on valinomycin.

The beauvericin-induced electrical conductance of an artificial lipid membrane (1,2-diphytanoyl-3-glycerophosphorylcholine in *n*-decane obtained from Dr. Steve Kennedy) has been studied in our laboratory using the method of Mueller and Rudin.[15] A full account is to be published elsewhere, but it is appropriate to summarize here our results with divalent cations. Both calcium and barium gave a second-order relationship between conductance and antibiotic concentration for both picrate and chloride salts. Beauvericin-induced conductance was better with barium picrate rather than calcium picrate, but better with calcium chloride rather than barium chloride.

The second-order concentration dependence strongly suggests the presence of two molecules of beauvericin in the transporting complex as was found in the crystal structure.

On the basis of the crystal-structure determination and our membrane transport observations we are proposing as an interesting exercise the following steps which will follow the $(\mathrm{BvBaPic_3BaBv})^+$ complex within the membrane to the membrane-water interface where the complex disassembles and the ions are discharged into the water phase. In some steps it will be necessary to specify the orientation of a molecular species at the membrane-water interface. This will be done by enclosing the formula of the molecule within brackets with W and M at the appropriate ends to specify the orientation. For example, a free fatty acid at a water-air interface would be given as ${}_{\mathrm{A}}[\mathrm{RCOOH}]_{\mathrm{W}}$. Molecules within a phase will be subscripted without the use of brackets.

The first step is the loss of one molecule of beauvericin from the complex.

$$(\mathrm{BvBaPic_3BaBv})_{\mathrm{M}}{}^+ \rightleftharpoons (\mathrm{BvBaPic_3Ba})_{\mathrm{M}}{}^+ + \mathrm{Bv_M} \tag{1}$$

The new species, being less lipid soluble, is more stable when oriented at the membrane-water interface.[16]

$$(\mathrm{BvBaPic_3Ba})_{\mathrm{M}}{}^+ \rightleftharpoons {}_{\mathrm{M}}[(\mathrm{BvBaPic_3Ba})^+]_{\mathrm{W}} \tag{2}$$

Ions are now progressively lost into the water.

$${}_{\mathrm{M}}[(\mathrm{BvBaPic_3Ba})^+]_{\mathrm{W}} \rightleftharpoons {}_{\mathrm{M}}[(\mathrm{BvBaPic_3})^-]_{\mathrm{W}} + \mathrm{Ba_W}{}^{2+} \tag{3}$$

$${}_{\mathrm{M}}[(\mathrm{BvBaPic_3})^-]_{\mathrm{W}} \rightleftharpoons {}_{\mathrm{M}}[(\mathrm{BvBaPic_2})]_{\mathrm{W}} + \mathrm{Pic_W}{}^- \tag{4}$$

$${}_{\mathrm{M}}[(\mathrm{BvBaPic_2})]_{\mathrm{W}} \rightleftharpoons {}_{\mathrm{M}}[(\mathrm{BvBaPic})^+]_{\mathrm{W}} + \mathrm{Pic_W}{}^- \tag{5}$$

$${}_{\mathrm{M}}[(\mathrm{BvBaPic})^+]_{\mathrm{W}} \rightleftharpoons {}_{\mathrm{M}}[(\mathrm{BvBa})^{2+}]_{\mathrm{W}} + \mathrm{Pic_W}{}^- \tag{6}$$

$${}_{\mathrm{M}}[(\mathrm{BvBa})^{2+}]_{\mathrm{W}} \rightleftharpoons {}_{\mathrm{M}}[\mathrm{Bv}]_{\mathrm{W}} + \mathrm{Ba_W}{}^{2+} \tag{7}$$

The free beauvericin can now return to the lipid environment of the membrane.

$${}_{\mathrm{M}}[\mathrm{Bv}]_{\mathrm{W}} \rightleftharpoons \mathrm{Bv_M} \tag{8}$$

Some of the intermediate forms as well as picrate ion may exist within the membrane also. However, based on our membrane-transport data thus far, these should not contribute more than a few percent to the overall transport of ions. We are presently starting a new series of lipid bilayer conductivity experiments intended to provide data for conductivity vs. cation and conductivity vs. anion concentrations, which should give much more information about the beauvericin-induced membrane transport of ions. Although picrate would not be present in vivo, comparison of the beauvericin-barium picrate with the "double-binding" calcium site in thermolysin[14] mentioned previously suggests that glutamate or aspartate could replace picrate.

Conclusions

The beauvericin-metal ion-anion molecular unit observed in more than one crystalline state suggests that it is a very stable association. Ion-transport studies through artificial membranes show second-order dependence of conductivity on beauvericin concentrations and anion-dependent cation specificity. These results suggest that the molecular cluster of the crystalline state is also the transporting species in solution.

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Supplementary Material Available: A table of observed and calculated structure factors (15 pages). Ordering information is given on any current masthead page.

References and Notes

(1) R. L. Hammill, C. E. Higgins, H. E. Boaz, and M. Gorman, *Tetrahedron Lett.*, **49**, 4255–4258 (1969).

(2) S. Estrada-O., C. Gomez-Louero, and M. Montal, *Bioenergetics*, **3**, 417–428 (1972).

(3) E. Dorschner and H. Lardy, *Antimicrob. Agents Chemother.*, 11–19 (1968).

(4) R. W. Roeske, S. Isaac, T. E. King, and L. K. Steinrauf, *Biochem. Biophys. Res. Commun.*, **57**, 554–561 (1974).

(5) J. M. Pinkerton and L. K. Steinrauf, *J. Org. Chem.*, **32**, 1828–1832 (1967).

(6) R. C. Prince, A. R. Crofts, and L. K. Steinrauf, *Biochem. Biophys. Res. Commun.*, **59**, 697–703 (1974).

(7) V. T. Ivanov, A. V. Evstratov, L. V. Sumskaya, E. I. Melnik, T. S. Chumburidze, S. L. Portnova, T. A. Balasheva, and Yu. A. Ovchinnikov, *FEBS Lett.*, **36**, 65–71 (1973).

(8) J. A. Hamilton, L. K. Steinrauf, and B. Braden, *Biochem. Biophys. Res. Commun.*, **64**, 151–156 (1975).

(9) A. J. Geddes, *Acta Crystallogr., Sect. B*, **32**, 3164–3171 (1976).

(10) "International Tables for X-ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1962, pp 202, 214.

(11) D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, **24**, 321–324 (1968).

(12) "The X-Ray System of Crystallographic Programs", J. Stewart, Ed., Technical Report TR-446, University of Maryland Computer Science Center, 1976.

(13) Y. A. Ovchinnikov, V. T. Ivanov, A. V. Estratov, I. I. Mikhaleva, V. F. Bystrov, S. L. Portnova, T. A. Balashova, E. N. Meshchenyakova, and V. M. Tulchinsky. *Int. J. Pept. Protein Res.*, **6**, 465–498 (1974).

(14) B. W. Mathews, L. H. Weaver, and W. R. Kester, *J. Biol. Chem.*, **249**, 8030–8044 (1974).

(15) P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26**, 398–404 (1967).

(16) L. K. Steinrauf and M. N. Sabesan, "Metal Ligand Interactions in Organic Chemistry and Biochemistry", Part 2, B. Pullman and N. Goldblum, Eds., D. Reidel Publishing Co., Dordrecht, Holland, pp 43–456.

(17) N. K. Dalley, D. W. Smith, R. M. Izatt, and J. J. Christiansen, *J. Chem. Soc., Chem. Commun.*, 90–91 (1972).

(18) D. Bright and M. R. Truter, *Nature* (*London*), **225**, 176–177 (1970).

(19) D. G. Davis and D. C. Tosteson, *Biochemistry*, 14, 3962–3969 (1975).

(20) K. Maartman-Moe, *Acta. Crystallogr., Sect. B*, **25**, 1452–1460 (1969).

(21) F. H. Herbstein, M. Kapon, and O. S. Wielinski, *Acta Crystallogr., Sect. B*, **33**, 649–654 (1977).

(22) U. Thewalt and C. E. Bugg, *Acta Crystallogr., Sect. B*, **28**, 82–92 (1972).

(23) D. L. Hughes, C. L. Mortimer, and M. R. Truter, *Inorg. Chim. Acta*, **28**, 83–89 (1978).

(24) J. A. Hamilton, M. N. Sabesan, B. F. Gisin, and L. K. Steinrauf, *Biochem. Biophys. Res. Commun.*, **80**, 949–954 (1978).

(25) J. A. Hamilton, M. N. Sabesan, and L. K. Steinrauf, in preparation.

(26) J. A. Hamilton, M. N. Sabesan, and L. K. Steinrauf, *Acta Crystallogr.*, in press.