Crystal Structure of Valinomycin-Sodium Picrate. Anion Effects on Valinomycin-Cation Complexes

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Abstract: The crystal structure of a complex of valinomycin with sodium picrate has been determined by single-crystal X-ray diffraction. The crystals were monoclinic, space group $P2_1$, with two molecules of valinomycin-sodium picrate per unit cell. Cell dimensions were as follows: a = 13.369 (7) Å, b = 21.908 (12) Å, c = 15.818 (8) Å, and β = 122.10 (1)°. The structure was determined by rotation-translation searches and refined by Fourier and least-squares methods. The final R value was 0.15 for all observed data. The structure revealed a water molecule in the region within valinomycin usually occupied by a potassium ion. The sodium ion was found to be external to the valinomycin, bonding to the three carbonyl oxygens adjacent to the lactyl residues and to the water molecule. The sodium also makes two bonds to the picrate anion. The sodium ion is thus displaced 2.3 Å from the position of the potassium ion as found in the crystal structure of valinomycin-potassium picrate. By comparison to potassium, sodium is more weakly bound to the valinomycin and more strongly bound to the picrate. This different binding can explain the much greater effects of picrate-like anions on the valinomycin-catalyzed membrane transport of sodium and the smaller effects on potassium transport. The results also explain the very different spectra shown by valinomycin-potassium and valinomycin-sodium complexes in solution.

Valinomycin is a cyclic dodecadepsipeptide containing residues of D-valine, D-α-hydroxyisovaleric acid, L-valine, and L-lactic acid in the sequence *cyclo*(L-val-D-hyv-D-val-L-lac)$_3$. Valinomycin is one of the most intensely studied of the ion-transporting antibiotics (ionophores). Crystal structure determinations have been published on the valinomycin-potassium aurichloride complex by Pinkerton, Steinrauf, and Dawkins,[1] on uncomplexed valinomycin by Smith et al.[2] and by Karle,[3] and on the valinomycin-potassium tri-/pentaiodide complex by Neupert-Laves and Dobler.[4] The crystal structure determination of a proline analogue of valinomycin—prolinomycin-rubidium picrate—has recently been published by Hamilton et al.[5]

Three distinct conformations are recognized for valinomycin. The bracelet (or octahedral) form simultaneously was postulated from solution studies by Ivanov et al.[6] and was observed in a crystal structure by Pinkerton et al.[1] This form is assumed to be the conformation for all monovalent cation complexes in both high polarity solvents and low polarity solvents and has been found in all crystal structures of monovalent cation complexes thus far. This conformation has six regularly spaced hydrogen bonds and, when complexed with potassium, has the cation in the center of an octahedral arrangement of oxygen atoms from the valyl residues. It is also assumed that this is a possible conformation for uncomplexed valinomycin in low polarity solvents. Ivanov et al.[6] also postulated a more open form in solvents of high polarity. In this form every other hydrogen bond has been opened and the neighboring carbonyl groups are rotated to point outward. Combinations of hydrogen bond breaking and rotations of residues have been assumed by Smith and Duax[7] in their proposed mechanism of cation capture by valinomcyin in solution. A third conformation, that of uncomplexed valinomycin, at this point found only in crystal structures,[2,3] has two of the six hydrogen bonds rearranged to involve the valyl carbonyl oxygens. Possibly yet another conformation of valinomycin is that in a crystal structure with barium thiocyanate which has been announced by Devarajan et al.,[8] but details have not been published.

Table I. Crystal Data of Sodium Valinomycin Picrate

a = 13.369 (7) Å	space group $P2_1$
b = 21.908 (12) Å	Z = 2
c = 15.818 (8) Å	V = 3925 Å^3
β = 122.1 (2)°	

Nuclear magnetic resonance has been used to probe the structure of valinomycin in solution,[9-11] and membrane transport studies have been carried out to examine the factors influencing its ion-transporting function.[12-16] In early studies on valinomycin the function of the anion was thought to be merely one of providing electroneutrality, but more recent investigations have demonstrated the influence of certain anions,[17-20] such as trinitrocresolate (3-methyl picrate) on the complexation and transport of sodium and potassium by valinomycin. With these observations in mind we have undertaken the crystal structure determinations of valinomycin-potassium picrate and valinomycin-sodium picrate; details of the potassium complex have been published.[21]

Two other crystal structures of picrate salts of antibiotic complexes have been determined. These are the proline analogue of valinomycin, prolinomycin-rubidium picrate,[5] in which the picrate

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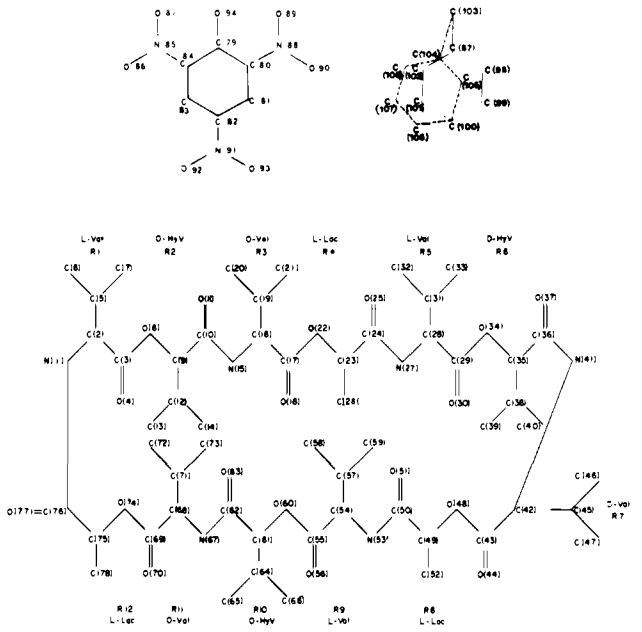

Figure 1. The numbering scheme of the Val molecule with the water molecule, the picrate ion, and the solvent molecule (xylene with one methyl missing and with the alternate numbering shown with the dotted lines).

is not close to the cation, and beauvericin–barium picrate,[22] in which is found the five-layered cluster (Bv.Ba.Pic3.Ba.Bv) with the three picrates forming a bridge between the cations and the other picrate being some distance away.

Experimental Section

Crystallization and Crystal Data. Valinomycin was a gift from Eli Lilly and Co. Picrate was used as the anion because of the similarity to trinitrocresolate and because picrate had been used in the other crystal structures. Picric acid from Fisher Scientific was neutralized in ethanol with an equivalent amount of sodium hydroxide. The product was recrystallized three times from hot ethanol. Crystals of valinomycin–sodium picrate were obtained from the slow evaporation of equimolar amounts of valinomycin and sodium picrate from a mixture of *m*-xylene and chloroform. This was the same procedure that produced valinomycin–potassium picrate crystals. Three-dimensional diffraction data consisting of 5322 nonzero unique reflections were collected at −170 °C on a Picker four-circle diffractometer in the Crystal Structure Center at Indiana University, Bloomington, IN. Of these 4528 had $F_o > 0$ and 3582 had $F_o > \sigma(F_o)$. The crystal used for data collection was chosen to minimize absorption errors; Mo Kα radiation was used, and no absorption corrections were made. Unit cell parameters at −170 °C are given in Table I.

Structure Determination and Refinement. A model for the structure was obtained by the same method that had been successful for the valinomycin–potassium picrate and described previously.[21] The coordinates of the valinomycin were found by moving the backbone atoms of valinomycin through the rotation–translation search as previously described. All other atoms of the structure were found by difference Fourier methods. Refinement was carried out by least-squares methods to a final *R* value of 0.15. The quantity minimized in the refinement was $\sum w(|F_o| - |F_c|)^2$, where $w = 1/\sigma(F_o)$. Scattering factors were taken from ref 23. All computer programs were from the X-RAY 76 system except those used for the rotation–translation searches which were written by us.

Results and Discussion

Molecular Structure. The numbering scheme for the valinomycin molecule is given in Figure 1. A stereo view of the structure is given in Figure 2. The final fractional coordinates and temperature parameters are listed in Table II. Bond lengths for the structure are listed in Table III and the corresponding bond angles in Table IV; the coordination distances involving the sodium are given in Table V and hydrogen bond distances and angles in Table VI. The torsional angles, compared with other valinomycin structures, are given in Table VII.

The conformation of the valinomycin molecule in this structure is only slightly changed from that in valinomycin–potassium picrate. The six carbonyl oxygens still point inward, and the six peptide nitrogens still hydrogen bond to the other carbonyl groups. The major changes are in the way in which the anion and cation are bound. The Fourier synthesis calculated on the basis of the valinomycin backbone atoms showed many other peaks, two of which were particularly heavy. One was at the expected position of the cation (sodium), and the other was about 2.3 Å away in the direction of the lactyl residues. An examination of peak density revealed the inner peak to be compatible with water and the outer peak with sodium. This assumption was verified by the successful refinement of the structure giving reasonable temperature factors and bond lengths and angles. This displacement of the sodium to the outside of the coordination cage of valinomycin was not expected nor was the presence of the water molecule. Although no water had been added to the preparation, no efforts were made to maintain water-free conditions. It is therefore assumed that the water had come from the atmosphere. Geddes had reported a similar experience with his crystal structure of uncomplexed beauvericin.[24]

The structure is actually valinomycin monohydrate–sodium picrate. The single molecule of water sitting within the valinomycin cavity makes a short bond to the sodium and contacts which must be hydrogen bonds to the three L-valyl carbonyl oxygens. With only two hydrogen atoms the water molecule obviously cannot bond to each of the three carbonyls. Quite possibly the hydrogen atoms are disordered; in some unit cells the hydrogen bonding is to O4 and to O30 and in others the bonding is to O4 and to O56. Bonding to O30 and to O56 from the same water molecule probably cannot take place because the O30–water–O56 angle is unfavorable. (See the hydrogen bonding distances and angles in Table VI.)

The sodium ion sits 0.24 Å above (as seen in Figure 2) the plane of the D-valyl carbonyl oxygens. The situation for the picrate anion is very different from that found for the valinomycin–potassium picrate structure in which the picrate made several van der Waals interactions with the L-valyl and D-isovaleryl residues and the para-nitro group made a weak interaction of 3.8 Å with the potassium. In the present structure the phenolate oxygen atom and one ortho-nitro oxygen atom make strong bonds to the sodium (Table V).

The picrate is more asymmetric in the sodium structure than in the potassium. Likewise the valinomycin molecule is more asymmetric (less threefold symmetry) in the sodium structure. This loss of symmetry in the sodium structure would be expected from the strong interaction between the valinomycin molecule and the picrate by which each is perturbing the shape of the other. This perturbation has been found in the CD spectra[10] of solutions of valinomycin–sodium trinitrocresolate in which there are strong Cotton effects in the absorption spectra of the trinitrocresolate, which demonstrates that the trinitrocresolate has been made asymmetric by the association with the valinomycin molecule.

The solvent molecule, *m*-xylene, is disordered and the second methyl group was never located. The xylene was fit by assuming two distinct positions related by pivoting about the methyl group and also having atoms C100 and C103 in common. The xylene is situated in much the same way as was found in the structure of valinomycin–potassium picrate albeit at the opposite end of the valinomycin molecule.

Correlations with Solution Studies. From the results of our studies we would predict, as is well-known, that valinomycin would complex potassium much better than sodium but also that valinomycin would stabilize the potassium picrate ion pair and would strongly stabilize the sodium picrate ion pair. There are numerous examples in the literature from the results of studies of valinomycin in solutions and in membrane systems which provide evidence of interactions with anions.

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Table II. Fractional Atomic Parameters with their Estimated Standard Deviations[a]

atom no.	x	y	z	U,[b] Å²	atom no.	x	y	z	U,[b] Å²
N(1)	−2684 (13)	678 (7)	−911 (11)	4.0 (4)	C(55)	2702 (16)	−470 (9)	2364 (13)	4.1 (5)
C(2)	−3174 (15)	65 (8)	−1267 (13)	3.6 (4)	O(56)	2087 (12)	−831 (7)	2449 (10)	5.3 (4)
C(3)	−2930 (15)	−292 (8)	−333 (13)	3.7 (4)	C(57)	4660 (17)	−916 (10)	3657 (15)	5.0 (5)
O(4)	−1933 (11)	−331 (6)	434 (9)	4.4 (3)	C(58)	4578 (16)	−1361 (9)	2881 (14)	3.9 (5)
C(5)	−2726 (15)	−251 (8)	−1809 (13)	3.5 (4)	C(59)	5954 (18)	−786 (10)	4459 (15)	5.3 (5)
C(6)	−3207 (16)	−941 (9)	−2087 (13)	3.9 (5)	O(60)	2393 (10)	−108 (6)	1579 (8)	3.7 (3)
C(7)	−3071 (16)	110 (9)	−2778 (14)	4.0 (5)	C(61)	1211 (14)	−165 (8)	735 (12)	3.3 (4)
O(8)	−3881 (10)	−590 (6)	−447 (9)	3.9 (3)	C(62)	655 (14)	446 (8)	431 (12)	3.4 (4)
C(9)	−3669 (17)	−947 (9)	392 (14)	4.6 (5)	O(63)	−330 (11)	520 (6)	−353 (9)	4.4 (3)
C(10)	−3682 (14)	−539 (8)	1191 (12)	3.2 (4)	C(64)	1267 (17)	−448 (9)	−171 (14)	4.3 (5)
O(11)	−3378 (10)	−793 (6)	1987 (9)	3.9 (3)	C(65)	2200 (18)	−67 (10)	−268 (16)	5.1 (5)
C(12)	−4586 (19)	−1457 (10)	55 (16)	5.6 (6)	C(66)	1610 (16)	−1119 (9)	49 (14)	4.2 (5)
C(13)	−5822 (23)	−1203 (13)	−512 (20)	7.8 (8)	N(67)	1251 (12)	941 (7)	1034 (10)	3.3 (3)
C(14)	−4428 (38)	−1947 (22)	−471 (33)	14.7 (16)	C(68)	653 (15)	1542 (8)	709 (12)	3.4 (4)
N(15)	−3998 (12)	38 (7)	953 (10)	3.7 (4)	C(69)	−492 (13)	1484 (7)	750 (11)	2.7 (4)
C(16)	−3908 (16)	412 (9)	1808 (13)	4.1 (4)	O(70)	−503 (11)	1323 (6)	1463 (9)	4.2 (3)
C(17)	−2633 (15)	401 (9)	2673 (13)	3.7 (4)	C(71)	1454 (16)	2042 (9)	1443 (14)	4.0 (5)
O(18)	−1756 (10)	481 (6)	2593 (9)	3.8 (3)	C(72)	804 (16)	2643 (9)	1225 (13)	3.8 (4)
C(19)	−4292 (15)	1084 (8)	1431 (13)	3.4 (4)	C(73)	2552 (16)	2124 (9)	1395 (14)	4.4 (5)
C(20)	−4166 (17)	1440 (9)	2328 (14)	4.4 (5)	O(74)	−1424 (10)	1700 (6)	−104 (8)	3.6 (3)
C(21)	−5614 (17)	1096 (9)	564 (15)	4.5 (5)	C(75)	−2516 (14)	1663 (8)	−156 (12)	3.1 (4)
O(22)	−2546 (10)	313 (5)	3535 (8)	3.4 (3)	C(76)	−3129 (14)	1043 (7)	−519 (12)	3.0 (4)
C(23)	−1393 (15)	343 (9)	4445 (13)	3.8 (4)	O(77)	−3956 (10)	913 (6)	−443 (8)	3.8 (3)
C(24)	−775 (14)	−295 (8)	4655 (12)	3.0 (4)	C(78)	−3342 (22)	2158 (12)	−953 (18)	7.0 (7)
O(25)	251 (10)	−301 (6)	5418 (8)	3.7 (3)	C(79)	909 (14)	2375 (8)	3537 (12)	3.2 (4)
C(26)	−1574 (17)	550 (10)	5248 (15)	4.7 (5)	C(80)	−282 (16)	2600 (9)	3074 (14)	4.4 (5)
N(27)	−1295 (12)	−758 (7)	4084 (10)	3.7 (3)	C(81)	−637 (17)	3206 (9)	2821 (14)	4.5 (5)
C(28)	−633 (16)	−1332 (9)	4319 (13)	3.9 (4)	C(82)	205 (14)	3633 (8)	3009 (12)	2.8 (4)
C(29)	469 (15)	−1213 (8)	4305 (13)	3.7 (4)	C(83)	1372 (15)	3470 (8)	3425 (12)	3.3 (4)
O(30)	499 (11)	−954 (6)	3648 (9)	4.2 (3)	C(84)	1713 (13)	2868 (7)	3716 (11)	2.4 (4)
C(31)	−1383 (14)	−1841 (8)	3555 (12)	3.1 (4)	N(85)	2957 (13)	2727 (7)	4201 (11)	3.9 (4)
C(32)	−603 (19)	−2429 (10)	3879 (16)	5.5 (6)	O(86)	3423 (12)	2439 (7)	4960 (10)	5.3 (4)
C(33)	−2486 (16)	−1955 (9)	3587 (13)	3.9 (4)	O(87)	3494 (13)	2901 (7)	3806 (11)	6.1 (4)
O(34)	1421 (11)	−1458 (6)	5093 (9)	4.5 (3)	N(88)	−1224 (15)	2160 (8)	2840 (13)	5.5 (5)
C(35)	2540 (17)	−1409 (10)	5122 (15)	4.9 (5)	O(89)	−1006 (14)	1706 (8)	3339 (12)	6.5 (5)
C(36)	3083 (17)	−796 (10)	5481 (14)	4.8 (5)	O(90)	−2214 (14)	2317 (8)	2122 (12)	7.2 (5)
O(37)	3956 (11)	−672 (6)	5400 (9)	4.2 (3)	N(91)	−93 (16)	4254 (9)	2764 (13)	6.1 (5)
C(38)	3373 (16)	−1918 (9)	5766 (13)	4.0 (4)	O(92)	647 (14)	4624 (8)	2883 (12)	6.8 (4)
C(39)	2822 (20)	−2543 (11)	5353 (17)	6.2 (6)	O(93)	−1142 (14)	4389 (8)	2435 (11)	6.5 (4)
C(40)	3744 (21)	−1822 (11)	6873 (18)	6.4 (6)	O(94)	1225 (14)	1841 (7)	3689 (10)	5.0 (3)
N(41)	2655 (11)	−390 (6)	5862 (10)	3.0 (3)	Na(95)	242 (6)	899 (4)	3042 (5)	*c*
C(42)	3242 (14)	206 (8)	6149 (12)	3.2 (4)	W(96)	202 (10)	0 (0)	2241 (8)	*c*
C(43)	3017 (14)	532 (8)	5218 (12)	3.4 (4)	C(97)	0	−2237	1614	5.3 (11)
O(44)	2071 (11)	572 (6)	4442 (9)	4.2 (3)	C(98)	1579	−2303	2296	6.6 (12)
C(45)	2689 (18)	585 (10)	6666 (15)	5.2 (5)	C(99)	1842	−2961	2500	9.4 (19)
C(46)	3060 (18)	281 (10)	7640 (16)	5.3 (5)	C(100)	1053	−3355	2477	9.6 (9)
C(47)	3110 (19)	1242 (10)	6785 (16)	5.2 (5)	C(101)	−263	−3290	2046	8.2 (15)
O(48)	4015 (9)	764 (5)	5362 (8)	3.4 (3)	C(102)	−790	−2763	1568	5.6 (11)
C(49)	3935 (14)	1094 (8)	4551 (12)	3.2 (4)	C(103)	−500	−1711	1364	9.5 (9)
C(50)	3830 (16)	683 (9)	3725 (13)	4.2 (4)	C(104)	−263	−2500	1614	4.6 (10)
O(51)	3574 (10)	925 (6)	2949 (9)	3.7 (3)	C(105)	895	−2763	2136	5.1 (10)
C(52)	5173 (19)	1454 (10)	5014 (16)	4.9 (5)	C(106)	−263	−3750	1932	8.9 (17)
N(53)	4053 (11)	77 (6)	3928 (10)	3.1 (3)	C(107)	−1316	−3421	1546	8.6 (16)
C(54)	3974 (15)	−314 (8)	3183 (13)	3.6 (4)	C(108)	−1316	−2829	1364	8.1 (16)

[a] The positional parameters have been multiplied by 10^4 and the thermal parameters by 10^2. [b] The isotropic temperature factor is of the form $T = \exp(-8\pi^2 U(\sin^2\theta)/\lambda^2)$ and the anisotropic temperature factor $T = \exp[-2\pi^2(h^2a^{*2}U(11) + k^2b^{*2}U(22) + l^2c^{*2}U(33) + 2hka^*b^*U(12) + 2hla^*c^*U(13) + 2klb^*c^*U(23)]$. [c] The anisotropic temperature of Na and W(96) are respectively as follows: $U(11) = 2.8$ (4), 3.4 (7); $U(22) = 5.6$ (5), 6.1 (9); $U(33) = 3.9$ (4), 3.0 (7); $U(12) = -0.7$ (4), 0.3 (7); $U(13) = 1.6$ (3), 1.4 (6); $U(23) = -0.1$ (4), 0.4 (6).

Tosteson and co-workers[19] have described the effects of valinomycin on the electrical conductance and isotope tracer-measured unidirectional cation fluxes of sodium and potassium trinitrocresolate (3-methyl picrate) across bilayers formed from sheep red blood cell lipids dissolved in decane. In the presence of trinitrocresolate but no valinomycin they found increased electrical conductance due to anion transport and increased cation fluxes due to a much greater rate of ion-pair transport. There was no significant discrimination between sodium and potassium. In the presence of valinomycin but no trinitrocresolate they found the electrical conductance and cation flux to be about 1000 times larger for potassium than for sodium. The addition of trinitrocresolate resulted in a greater increase in the conductance and cation flux of sodium than of potassium until at about 0.01 M trinitrocresolate the cation discrimination had been abolished. This can be explained most easily by a strong interaction between the trinitrocresolate and the valinomycin–sodium complex. However, the above studies do not suggest any mechanism for the interaction of the anion.

The bracelet conformation of valinomycin complexes has a pocket in each end. The ability of xylene to occupy an end of the valinomycin molecule, and in particular the penetration of one methyl group into the cavity suggests that anions could be classified according to their lipophilic nature and according to their ability to penetrate into the cavities at the ends of valinomycin. In this respect the two oxygen atoms of a nitro group are somewhat too wide apart; a single electronegative atom or linear group of atoms would be much more effective in making close contact with the cation in the interior of valinomycin. An example of this is provided by the work of Kuo et al.[25] on the blocking of valino-

Table III. Intramolecular Bond Lengths (Å) and Standard Deviations

Valinomycin Molecule			
N(1)-C(2)	1.47 (2)	C(38)-C(39)	1.53 (3)
N(1)-C(76)	1.33 (3)	C(38)-C(40)	1.56 (3)
C(2)-C(3)	1.55 (3)	N(41)-C(42)	1.46 (2)
C(2)-C(5)	1.46 (3)	C(42)-C(43)	1.52 (3)
C(3)-O(4)	1.24 (2)	C(42)-C(45)	1.60 (4)
C(3)-O(8)	1.35 (3)	C(43)-O(44)	1.21 (2)
C(5)-C(6)	1.61 (3)	C(43)-O(48)	1.33 (2)
C(5)-C(7)	1.56 (3)	C(45)-C(46)	1.50 (3)
O(8)-C(9)	1.43 (3)	C(45)-C(47)	1.52 (3)
C(9)-C(10)	1.56 (3)	O(48)-C(49)	1.43 (2)
C(9)-C(12)	1.53 (3)	C(49)-C(50)	1.53 (3)
C(10)-O(11)	1.23 (2)	C(49)-C(52)	1.62 (3)
C(10)-N(15)	1.32 (2)	C(50)-O(51)	1.21 (2)
C(12)-C(13)	1.51 (3)	C(50)-N(53)	1.36 (2)
C(12)-C(14)	1.44 (6)	N(53)-C(54)	1.42 (3)
N(15)-C(16)	1.53 (3)	C(54)-C(55)	1.53 (2)
C(16)-C(17)	1.52 (2)	C(54)-C(57)	1.55 (3)
C(16)-C(19)	1.57 (3)	C(55)-O(56)	1.20 (3)
C(17)-O(18)	1.26 (3)	C(55)-O(60)	1.34 (2)
C(17)-O(22)	1.32 (3)	C(57)-C(58)	1.53 (3)
C(19)-C(20)	1.55 (3)	C(57)-C(59)	1.53 (3)
C(19)-C(21)	1.56 (2)	O(60)-C(61)	1.43 (2)
O(22)-C(23)	1.45 (2)	C(61)-C(62)	1.48 (2)
C(23)-C(24)	1.57 (3)	C(61)-C(64)	1.60 (3)
C(23)-C(26)	1.48 (4)	C(62)-O(63)	1.25 (2)
C(24)-O(25)	1.26 (2)	C(62)-N(67)	1.38 (2)
C(24)-N(27)	1.29 (2)	C(64)-C(65)	1.58 (4)
N(27)-C(28)	1.47 (2)	C(64)-C(66)	1.53 (3)
C(28)-C(29)	1.51 (3)	N(67)-C(68)	1.48 (2)
C(28)-C(31)	1.56 (2)	C(68)-C(69)	1.57 (3)
C(29)-O(30)	1.20 (3)	C(68)-C(71)	1.54 (2)
C(29)-O(34)	1.33 (2)	C(69)-O(74)	1.35 (2)
C(31)-C(32)	1.56 (3)	C(69)-O(70)	1.19 (3)
C(31)-C(33)	1.52 (3)	C(71)-C(72)	1.51 (3)
O(34)-C(35)	1.48 (3)	C(71)-C(73)	1.52 (4)
C(35)-C(36)	1.49 (3)	O(74)-C(75)	1.42 (3)
C(35)-C(38)	1.52 (3)	C(75)-C(76)	1.53 (2)
C(36)-O(37)	1.27 (3)	C(75)-C(78)	1.58 (3)
C(36)-N(41)	1.36 (3)	C(76)-O(77)	1.21 (3)
Xylene Molecules			
C(97)-C(98)	1.79	C(104)-C(103)	1.77
C(97)-C(102)	1.54	C(104)-C(105)	1.43
C(97)-C(103)	1.29	C(104)-C(108)	1.44
C(98)-C(99)	1.48	C(105)-C(100)	1.38
C(99)-C(100)	1.35	C(100)-C(106)	1.72
C(100)-C(101)	1.52	C(106)-C(107)	1.40
C(101)-C(102)	1.35	C(107)-C(108)	1.33
Picrate Anion			
C(79)-C(80)	1.44 (3)	C(83)-C(84)	1.39 (2)
C(79)-C(84)	1.44 (3)	C(84)-N(85)	1.45 (2)
C(79)-O(94)	1.22 (2)	N(85)-O(86)	1.20 (2)
C(80)-C(81)	1.40 (3)	N(85)-O(87)	1.24 (3)
C(80)-N(88)	1.47 (3)	N(88)-O(89)	1.21 (3)
C(81)-C(82)	1.37 (3)	N(88)-O(90)	1.25 (2)
C(82)-C(83)	1.38 (2)	N(91)-O(92)	1.21 (3)
C(82)-N(91)	1.41 (3)	N(91)-O(93)	1.24 (3)

mycin-mediated conductance by substituted benzimidazoles. The best blocking agent that they found was 4,5,6,7-tetrachloro-2-(trifluoromethyl)benzimidazole in its anionic form. The minimum conductance was obtained sharply at 1:1 valinomycin:benzimidazole anion ratio, while those benzimidazoles which were the weaker blocking agents required higher concentrations.

Tosteson and co-workers had not investigated the range in which the concentration of the antibiotic was equal to that of the anion nor did Ginsberg and Stark,[20] who studied the effects of picrate and dinitrophenolate on the electrical conductance of lipid bilayers in the presence and absence of valinomycin and nonactin. They too found that picrate was able to abolish the cation specificity of valinomycin and that it increased the conductance in both the

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Table IV. Bond Angles (Deg) with Standard Deviations

Valinomycin Molecule			
C(2)-N(1)-C(76)	121 (2)	C(35)-C(38)-C(40)	110 (2)
N(1)-C(2)-C(3)	106 (1)	C(39)-C(38)-C(40)	113 (2)
N(1)-C(2)-C(5)	115 (2)	C(36)-N(41)-C(42)	116 (2)
C(3)-C(2)-C(5)	112 (2)	C(43)-C(42)-C(45)	110 (1)
C(2)-C(3)-O(4)	123 (2)	N(41)-C(42)-C(43)	109 (1)
C(2)-C(3)-O(8)	114 (1)	N(41)-C(42)-C(45)	108 (2)
O(4)-C(3)-O(8)	123 (2)	C(42)-C(43)-O(48)	111 (1)
C(2)-C(5)-C(6)	112 (2)	O(44)-C(43)-O(48)	124 (2)
C(2)-C(5)-C(7)	110 (2)	C(42)-C(45)-C(46)	108 (2)
C(6)-C(5)-C(7)	110 (1)	C(42)-C(45)-C(47)	108 (2)
C(3)-O(8)-C(9)	115 (1)	C(46)-C(45)-C(47)	113 (2)
O(8)-C(9)-C(10)	111 (2)	C(43)-O(48)-C(49)	117 (1)
O(8)-C(9)-C(12)	110 (1)	O(48)-C(49)-C(50)	113 (1)
C(10)-C(9)-C(12)	111 (2)	O(48)-C(49)-C(52)	106 (1)
C(9)-C(10)-O(11)	115 (2)	O(50)-C(49)-C(52)	106 (2)
C(9)-C(10)-N(15)	117 (2)	C(49)-C(50)-O(51)	117 (2)
O(11)-C(10)-N(15)	127 (2)	C(49)-C(50)-N(53)	118 (2)
C(9)-C(12)-C(13)	111 (2)	O(51)-C(50)-N(53)	125 (2)
C(9)-C(12)-C(14)	114 (3)	C(50)-N(53)-C(54)	119 (2)
C(13)-C(12)-C(14)	113 (2)	N(53)-C(54)-C(55)	113 (2)
C(10)-N(15)-C(16)	113 (2)	N(53)-C(54)-C(57)	110 (1)
N(15)-C(16)-C(17)	108 (2)	C(55)-C(54)-C(57)	109 (1)
N(15)-C(16)-C(19)	108 (2)	C(54)-C(55)-O(56)	125 (2)
C(17)-C(16)-C(19)	110 (1)	C(54)-C(55)-O(60)	108 (2)
C(16)-C(17)-O(18)	125 (2)	O(56)-C(55)-O(60)	127 (1)
C(16)-C(17)-O(22)	112 (2)	C(54)-C(57)-C(58)	112 (2)
O(18)-C(17)-O(22)	123 (1)	C(54)-C(55)-C(59)	111 (2)
C(16)-C(19)-C(20)	106 (2)	C(58)-C(57)-C(59)	111 (2)
C(16)-C(19)-C(21)	110 (1)	C(55)-O(60)-C(61)	117 (2)
C(20)-C(19)-C(21)	109 (2)	O(60)-C(61)-C(62)	110 (1)
C(17)-O(22)-C(23)	119 (2)	O(60)-C(61)-C(64)	108 (2)
O(22)-C(23)-C(24)	109 (1)	C(62)-C(61)-C(64)	108 (2)
O(22)-C(23)-C(26)	107 (2)	C(61)-C(62)-O(63)	122 (1)
C(24)-C(23)-C(26)	113 (2)	C(61)-C(62)-N(67)	119 (1)
C(23)-C(24)-O(25)	113 (1)	O(63)-C(62)-N(67)	120 (2)
C(23)-C(24)-N(27)	122 (1)	C(61)-C(64)-C(65)	107 (2)
O(25)-C(24)-N(27)	125 (2)	C(61)-C(64)-C(66)	109 (2)
C(24)-N(27)-C(28)	118 (1)	C(65)-C(64)-C(66)	112 (2)
N(27)-C(28)-C(29)	108 (2)	C(62)-N(67)-C(68)	117 (1)
N(27)-C(28)-C(31)	111 (1)	N(67)-C(68)-C(69)	106 (2)
C(29)-C(28)-C(31)	110 (2)	N(67)-C(68)-C(71)	110 (1)
C(28)-C(29)-O(30)	125 (1)	C(69)-C(68)-C(71)	109 (2)
C(28)-C(29)-O(34)	112 (2)	C(68)-C(69)-O(70)	124 (1)
O(30)-C(29)-O(34)	123 (2)	C(68)-C(69)-O(74)	109 (2)
C(28)-C(31)-C(32)	107 (1)	O(70)-C(69)-O(74)	126 (2)
C(28)-C(31)-C(33)	109 (2)	C(68)-C(71)-C(72)	111 (1)
C(32)-C(31)-C(33)	110 (2)	C(68)-C(71)-C(73)	110 (2)
C(29)-O(34)-C(35)	117 (2)	C(72)-C(71)-C(73)	110 (2)
O(34)-C(35)-C(36)	111 (2)	C(69)-O(74)-C(75)	114 (2)
O(34)-C(35)-C(38)	110 (2)	O(74)-C(75)-C(76)	114 (2)
C(36)-C(35)-C(38)	112 (1)	O(74)-C(75)-C(78)	105 (2)
C(35)-C(36)-O(37)	116 (2)	C(76)-C(75)-C(78)	108 (1)
C(35)-C(36)-N(41)	122 (2)	C(75)-C(76)-O(77)	121 (2)
C(37)-C(36)-N(41)	122 (2)	C(75)-C(76)-N(1)	115 (2)
C(35)-C(38)-C(39)	111 (1)	N(1)-C(76)-O(77)	124 (2)
Picrate Anion			
C(80)-C(79)-C(84)	111 (2)	C(83)-C(84)-N(85)	117 (2)
C(80)-C(79)-O(94)	127 (2)	C(79)-C(84)-C(85)	118 (1)
C(84)-C(79)-O(94)	122 (2)	C(84)-N(85)-O(86)	119 (2)
C(79)-C(80)-C(81)	126 (2)	C(84)-C(85)-O(87)	119 (1)
C(79)-C(80)-N(88)	118 (2)	O(86)-N(85)-O(87)	122 (2)
C(81)-C(80)-N(88)	116 (2)	C(80)-N(88)-O(89)	120 (1)
C(80)-C(81)-C(82)	118 (2)	C(80)-N(88)-O(90)	114 (2)
C(81)-C(82)-C(83)	121 (2)	C(89)-N(88)-O(90)	126 (2)
C(81)-C(82)-N(91)	121 (2)	C(82)-N(91)-O(92)	121 (2)
C(83)-C(82)-N(91)	118 (2)	C(82)-N(91)-O(93)	116 (2)
C(82)-C(83)-C(84)	119 (2)	O(92)-N(91)-O(93)	123 (2)
C(83)-C(84)-C(79)	124 (1)		
Xylene Molecules			
C(98)-C(97)-C(103)	121	C(103)-C(104)-C(105)	122
C(102)-C(97)-C(103)	115	C(103)-C(104)-C(108)	113
C(98)-C(97)-C(102)	122	C(105)-C(104)-C(108)	124
C(97)-C(98)-C(99)	106	C(100)-C(105)-C(104)	120
C(98)-C(99)-C(100)	122	C(105)-C(100)-C(106)	112
C(99)-C(100)-C(101)	131	C(100)-C(106)-C(107)	119
C(100)-C(101)-C(102)	118	C(106)-C(107)-C(108)	119
C(97)-C(102)-C(101)	118	C(104)-C(108)-C(107)	123

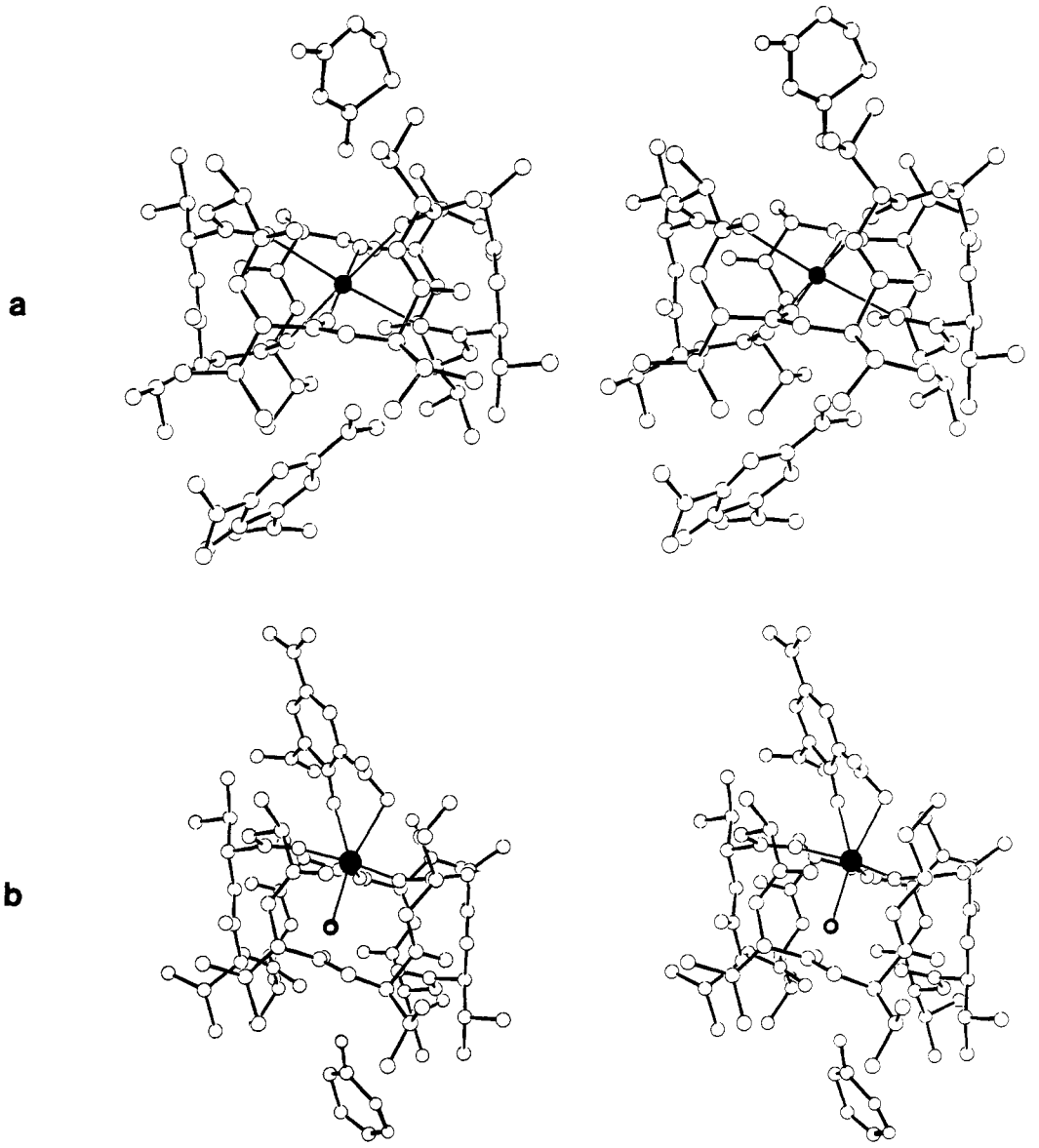

Figure 2. A stereoview of (a) the Val–potassium picrate xylene complex and (b) the Val–water–sodium picrate xylene complex. The water molecule is represented by the heavy circle. In each view the Val molecule has been given the same orientation and the cation is represented by the solid circle.

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

molecule	coordinating atoms	distance
valinomycin	Na(95)···O(18)	2.54 (2)
	Na(95)···O(44)	2.38 (1)
	Na(95)···O(70)	2.33 (2)
picrate	Na(95)···O(89)	2.64 (2)
	Na(95)···O(94)	2.37 (2)
water	Na(95)···W(96)	2.33 (1)

Table VI. Intramolecular Hydrogen Bond Lengths (Å) and Angles (Deg) of the Sodium and Potassium[21] Complexes of Valinomycin Picrate

	length		C–O···X angle	
hydrogen bond	K^+	Na^+	K^+	Na^+
N(1)–H···O(63)	2.88	2.80	135	138
N(15)–H···O(77)	2.89	2.95	135	128
N(27)–H···O(11)	2.85	2.99	145	137
N(41)–H···O(25)	2.87	2.91	132	137
N(53)–H···O(37)	2.85	2.91	136	130
N(67)–H···O(51)	2.82	2.97	137	128
W(96)–H···O(4)		2.86[a]		161
W(96)–H···O(30)		2.92[a]		160

[a] The water molecule exists only in the sodium structure.

presence and absence of valinomycin. They, however, chose to propose a model in which there is no direct connection between the anion and the valinomycin–cation complex. Instead, they speculated that picrate anions enhance the potassium transport by valinomycin by absorbing to the membrane–water interface and by generating a negative surface potential and that the picrate anions are passed through the membrane by means of dislocations of membrane lipid molecules caused by the passage of the valinomycin complexes. We, however, have shown that picrate does associate strongly with the valinomycin–sodium complex and more moderately with the valinomycin–potassium complex. A more valid test of their hypothesis should be based on whether or not valinomycin will increase the flux of a molecule with which it does not associate.

As revealed by crystal structure determinations[2,3] the uncomplexed form of valinomycin is flattened into an oval shape, loosing the threefold symmetry. No water molecules have been found in any of the uncomplexed forms. On the other hand the *cyclo*-hexadepsipeptide beauvericin is much more rigid than is valinomycin and does not collapse in the uncomplexed state; the crystal structure of uncomplexed beauvericin[24] was found to have water molecules in the center of the antibiotic. The binding at the ends of valinomycin–cation complexes must be stronger than that of uncomplexed valinomycin, partly due to the electrostatic field of the cation and partly due to the maintenance of a pocket

Table VII. Conformation Angles (Ramachandran and Sasisekharan, 1968)[27] of the Valinomycin Complexes and Uncomplexed Valinomycin[a]

conformation angle	complex	L-Val			D-Hyv			D-Val			L-Lac			ref
		R1	R5	R9	R2	R6	R10	R3	R7	R11	R4	R8	R12	
ϕ	$KAuCl_4$	−51	−64	−77	79	86	75	41	60	47	−81	−89	−79	1
	$RbAuCl_4$	−67	−60	−62	91	79	70	49	56	62	−85	−82	−91	1
	KI_5	−58	−60	−45	79	86	79	57	58	59	−66	−73	−76	4
	uncomplexed	−63	−108	−67	96	146	82	63	60	108	−74	−98	−164	3
	uncomplexed	−68	−110	−65	99	150	77	68	63	104	−75	−96	−162	3
	uncomplexed	−67	−110	−71	98	147	81	67	54	105	−71	−100	−165	2
	uncomplexed	−66	−108	−59	94	147	78	67	64	108	−71	−97	−166	2
	uncomplexed	−64	−102	−63	98	145	80	65	65	106	−77	−98	−160	2
	K picrate	−61	−59	−60	76	82	76	58	56	57	−86	−80	−77	21
	Na picrate	−64	−59	−77	81	81	130	60	66	64	−86	−77	−85	*b*
ψ	$KAuCl_4$	121	135	155	41	5	4	−113	−153	−132	−15	24	−3	1
	$RbAuCl_4$	131	137	142	4	11	11	−130	−137	−132	−9	−9	−2	1
	KI_5	131	133	133	3	−5	8	−129	−131	−133	−25	−16	−12	4
	uncomplexed	129	78	130	−8	−11	3	−134	−135	−69	−6	14	23	3
	uncomplexed	130	78	132	−3	−12	8	−134	−134	−71	−11	6	27	3
	uncomplexed	130	80	132	−6	−7	3	−136	−133	−68	−11	13	31	2
	uncomplexed	129	78	132	−5	−10	7	−136	−134	−68	−9	7	22	2
	uncomplexed	128	74	131	−4	−8	2	−134	−135	−71	−7	10	21	2
	K picrate	131	135	140	21	4	10	−136	−135	−139	−4	−6	−10	21
	Na picrate	131	135	140	21	4	10	−136	−135	−139	−4	−6	−10	*b*
ω	$KAuCl_4$	160	173	168	174	176	−174	173	−178	−172	180	172	168	1
	$RbAuCl_4$	−175	−175	−179	−172	180	177	167	−166	−164	176	176	−171	1
	KI_5	175	177	173	−179	−180	180	−177	−176	−174	−178	179	178	4
	uncomplexed	174	176	179	−179	172	180	−178	−172	−172	174	173	−178	3
	uncomplexed	174	174	−178	−179	172	−172	−178	−174	−173	174	177	178	3
	uncomplexed	174	174	177	173	179	177	173	−171	179	−171	−176	179	2
	uncomplexed	174	180	180	179	179	176	171	−172	−177	−172	−173	179	2
	uncomplexed	173	174	−179	174	180	174	169	−170	−170	−163	−164	180	2
	K picrate	179	174	172	174	−176	−177	−173	−173	−171	178	−179	179	21
	Na picrate	177	176	−178	−176	−178	−179	−175	−179	179	178	−179	179	*b*
χ^1	$KAuCl_4$	−43	−75	−79	175	60	77	59	65	36				1
	$RbAuCl_4$	−56	−55	−61	167	50	59	66	60	63				1
	KI_5	−64	−60	−61	166	62	70	65	63	64				4
	uncomplexed	−62	−62	−64	164	160	76	57	68	62				3
	uncomplexed	−67	−64	−65	166	164	66	66	68	63				3
	uncomplexed	−68	−70	−66	166	166	74	63	58	61				2
	uncomplexed	−61	−68	−62	162	164	68	68	64	57				2
	uncomplexed	−58	−64	−62	63	162	66	72	63	64				2
	K picrate	−56	−62	−68	179	173	173	64	61	69				21
	Na picrate	−63	−60	−57	73	59	72	64	68	66				*b*
χ^2	$KAuCl_4$	−175	177	158	−72	145	−53	180	176	−177				1
	$RbAuCl_4$	−174	177	151	−67	138	−81	179	−176	−178				1
	KI_5	172	180	179	−62	−58	−66	−173	−175	−173				4
	uncomplexed	179	177	177	−68	−64	−49	−178	−178	−177				3
	uncomplexed	179	175	174	−75	−60	−51	161	−176	−177				3
	uncomplexed	176	174	178	−58	−50	−74	−178	−178	−174				2
	uncomplexed	179	169	−178	−61	−54	−71	−174	−175	178				2
	uncomplexed	180	178	177	−59	−47	−69	−173	−178	177				2
	K picrate	−173	179	173	58	51	66	−178	−175	−175				21
	Na picrate	175	−179	179	−56	−66	−49	−179	−169	−171				*b*

[a] The residues (R1–R12) related by the molecular symmetry (e.g., R1, R5, R9) are grouped together for convenience. The convention for conformation angles is that proposed by the IUPAC-IUB Commission on biochemical nomenclature; Biochemistry 9:3471 (1970). [b] Present structure.

of appropriate size and shape at each of the two ends. The electrostatic field of the cation would be shielded by the polypeptide chain of valinomycin along the circumference and unshielded at the two ends. Stronger binding by complexed valinomycin than by uncomplexed was the conclusion reached by Levitt et al.,[26] who measured the electroosmotic volume flux and the open circuit streaming potential from osmotic pressure differences across lipid bilayers doped with valinomycin, nonactin, or gramicidin. They interpreted their observations as demonstrating the net flow of approximately four molecules of water in the direction of flow of the valinomycin–cation complex. Looking at the crystal structures of the valinomycin–cation complexes it is easy to visualize water molecules in each end of the complex, the water molecules hydrogen bonding together and forming at each end at least one coordination to the potassium.

Nuclear magnetic resonance studies by Davis and Tosteson[10] provide evidence in solution for the interactions of anions with cation complexes of valinomycin. In solutions of low polarity they found very little change in the α-C proton lines with different anions of potassium or rubidium complexes. Lithium and most salts of sodium gave complexes that were different from or possibly intermediate between the potassium complex and uncomplexed valinomycin; the cesium complex was also different. Sodium tetraphenylborate on the other hand gave a complex with valinomycin that was considered to be nearly identical with that of potassium salts. These results can be interpreted as demonstrating the external binding of sodium by valinomycin when an anion is available that can provide additional ligands to the cation. Tetraphenylborate cannot do this and in such case the sodium is probably entirely inside the valinomycin at the potassium binding position. The NH proton shifts were all found to be about the same except for uncomplexed valinomycin and for the cesium and lithium complexes. Results from carbon-13 NMR spectroscopy

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showed that the sodium ion was less effective than potassium in polarizing the carbonyl groups. Moreover, it was suggested that sodium interacts asymmetrically with valinomycin. Davis and Tosteson also investigated the circular dichroism spectrum of complexes of valinomycin in the region of the trinitrocresolate absorptive bands. They found small Cotton transitions with the potassium complex but different and much larger effects for sodium complexes, suggesting that the trinitrocresolate is much more closely associated with the valinomycin–sodium complex than with potassium and that the two cations have different interactions with the anion. Their kinetic and temperature-dependent studies revealed a more rapid dissociation rate for sodium complexes and a lower activation energy. It was their prediction that for sodium complexes of valinomycin with anions such as bromide, thiocyanate, or trinitrocresolate there is coordination of the sodium to only three of the six valyl carbonyl groups with other coordination coming from the anion. In view of our present results, this prediction has proven to be remarkably accurate.

The principal differences in the membrane transport properties of valinomycin and prolinomycin has been shown to be the rate at which the uncomplexed form of prolinomycin returns to pick up another cation and that prolinomycin undergoes complexation and release in solution rather than at the interface. From our crystal structure of prolinomycin–rubidium picrate we found no indications that the complex will bind picrate or the solvent (toluene or chloroform) in the crystal, while the prolinomycin complex was in the pseudosixfold conformation that we had found for valinomycin in the complexed form. We had previously suggested that the slower rate of cation transport by prolinomycin may be due to fewer degrees of rotational freedom of the prolyl residues resulting in the processes of complexation and release being slower. We can now also suggest that the prolyl residues form a less effective pocket at the ends of prolinomycin for the binding of other molecules. This could decrease the rate at which cations would find their way into the center of prolinomycin. Because of the poorly formed pocket prolinomycin may also be less able to orient properly at the interface so that pocket is facing outward into the aqueous solution.

Conclusions

Our structure determinations have shown a quite different binding by valinomycin for sodium than for potassium. The sodium-complexed form provides strong bonds to the anion which are not available from the potassium-complexed form. We would like to suggest that the ends of the valinomycin–potassium complex can bind water molecules and other electron-pair donors of appropriate shape and can help transport these through the lipid membrane. The valinomycin complex with sodium requires a lipophilic anion with electron pair donating capabilities in order to be stable. Most of the cation flux through the membrane is carried by the electrically neutral valinomycin–sodium–anion complex which should also have some abilities to help other neutral molecules through the membrane.

The structure of the valinomycin–water–sodium picrate complex also suggests that the structure of the uncomplexed valinomycin in the membrane may be that of the pseudosixfold symmetry form of six hydrogen bonds with a water molecule in the center rather than a cation. Therefore that form found in the crystal structures of uncomplexed valinomycin would not be a necessary intermediate. The cation capture could be accomplished at the interface by a loosening of one end of the valinomycin with about 0.5-Å outward displacement of the carbonyl groups of probably the lactyl end, loss of the water molecule through the slightly opened end, into the water solution, and replacement by a potassium or rubidium cation. This mechanism requires no major rearrangement of the polypeptide chain nor of the hydrogen bonding.

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Registry No. Na(L-Val-D-Hyv-D-Val-L-Lac)$_3$·picrate, 82093-46-7.

Supplementary Material Available: A table of observed and calculated structure factors (29 pages). Ordering information is given on any current masthead page.