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The Structure of Prolinomycin – a Synthetic Peptide Analog of Valinomycin

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Abstract

The crystal structure of prolinomycin rubidium picrate ($C_{60}H_{96}N_{12}O_{12}.Rb^+.C_6H_2N_3O_7^-.1\frac{1}{2}C_7H_8.CHCl_3$) has been determined using a rotation–translation search method. The conformation was found to be similar to that of valinomycin. The complex crystallizes in the triclinic system with two prolinomycin molecules and two rubidium cations and two picrate anions in a unit cell of dimensions a = 16·139 (11), b = 16·312 (9), c = 18·270 (11) Å, α = 106·70 (3), β = 86·95 (3), γ = 106·70 (3)°, space group $P\bar{1}$, Z = 2. The conformations of the two crystallographically independent prolinomycin molecules in the unit cell are very similar. Potential-energy calculations predict tighter binding of the cation and fewer low-energy uncomplexed conformations for prolinomycin as opposed to valinomycin, which explains the differences found in ion transport using artificial membranes.

Introduction

Prolinomycin (also called peptide PV) is a synthetic proline analog of valinomycin, *cyclo*-(-D-Val-L-Pro-L-Val-D-Pro-)$_3$. It was synthesized by Gisin & Merrifield (1972). Previous structural studies on valinomycin (Ivanov *et al.*, 1969; Pinkerton, Steinrauf & Dawkins, 1969) had suggested that the geometry of the ester linkages in valinomycin would be little changed by replacement with peptide linkages and that the solubility in nonaqueous solvents would be preserved as long as the peptide H atoms were not exposed to the exterior. In prolinomycin the ester linkage is replaced by a hydrogenless peptide linkage of the same chirality furnished by the proline residues. A series of studies (Gisin & Davis, 1973; Ting-Beall, Tosteson, Gisin & Tosteson, 1974; Davis, Gisin & Tosteson, 1976; Benz, Gisin, Ting-Beall, Tosteson & Lauger, 1976) showed that prolinomycin complexes monovalent cations more strongly than valinomycin but transports them more slowly through lipid bilayer membranes. A conformation was proposed for prolinomycin on the basis of these experiments.

A preliminary report of the results, described here in detail, has been published (Hamilton, Sabesan, Gisin & Steinrauf, 1978).

Experimental

A sample of prolinomycin was provided by Dr Balz Gisin. The complex was prepared by dissolving rubidium picrate with an equimolar amount of prolinomycin in methylene chloride and evaporating to dryness. Recrystallization from a 1:1 mixture of chloroform:toluene gave crystals suitable for X-ray diffraction. Since the crystals dried out on exposure to air, a single crystal was sealed in a thin-walled capillary tube with mother liquor present. The observed density was 1·27 (4) Mg m^{-3}; the density calculated for the unit cell containing two prolinomycin molecules, two Rb atoms and two picrate anions was considerably lower (1·13 Mg m^{-3}), suggesting the presence of solvent molecules. The calculated density including the solvents (three toluenes and two chloroforms) as found from the structure determination was 1·31 Mg m^{-3}. Accurate cell dimensions (Table 1) were obtained by the least-squares refinement of angular settings on a Hilger & Watts four-circle automated diffractometer. Complete three-dimensional data consisting of 7240 unique reflections were measured and it was noted that reflections with Miller indices $h + k + l$ odd were, in general, weak but present. All data collection was carried out in the laboratory of Dr Herman Watson, Department of Biochemistry, University of Bristol, England. All computer programs used, except those for the initial data processing, the rotation function and

Table 1. *Crystal data*

a = 16·139 (11) Å	Z = 2
b = 16·312 (9)	V = 4410 Å^3
c = 18·270 (11)	Space group $P\bar{1}$
α = 106·70 (3)°	M_r = 1749
β = 86·95 (3)	Number of independent reflections 7240
γ = 106·70 (3)	λ = 1·542 Å
	No absorption corrections were applied

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skewed Fourier synthesis, were from the XRAY system (1976).

Structure determination and refinement

Prolinomycin has a sequence which exhibits $\bar{1}$ symmetry. This means that the molecule can crystallize in a centrosymmetric space group through internal racemization. The choice of $P\bar{1}$, instead of $P1$, was therefore made. Statistical calculation on the E values also showed a centrosymmetric distribution. Furthermore, the Patterson map yielded a peak at $\frac{1}{2},\frac{1}{2},\frac{1}{2}$ corresponding to the Rb–Rb vector, which was consistent with a choice of $P\bar{1}$ as space group. The pseudo body-centering of the Rb atoms (at 0,0,0 and $\frac{1}{2},\frac{1}{2},\frac{1}{2}$) complicated the use of the statistical distribution of the reflections to solve the space-group ambiguity and rendered the Rb atom positions useless for initial phasing calculations (the Rb atoms contributed only to the phases of the $h + k + l$ even reflections). A Fourier map based on the Rb phases was calculated to see if any part of the structure could be identified and used to break the false symmetry but it was completely uninterpretable.

Attempts were made to find a possible starting set in order to try direct methods for structure determination but again the highest E values had indices of the class $h + k + l$ even and it was not possible to find triplets which would relate the two classes of reflections.

The crystal structure of valinomycin potassium tetrachloroaurate(III) (Pinkerton, Steinrauf & Dawkins, 1969) had shown the presence of a pseudo threefold axis in the valinomycin molecule. Prolinomycin is also a trimer and it seemed reasonable to assume that there should be fairly good threefold molecular symmetry, as was inferred from NMR results (Davis *et al.*, 1976). This led us to consider rotation–translation search methods to try to solve the structure. These methods had proved successful in solving the crystal structures of some complexes of cycloheptaamylose (Hamilton, Sabesan, Steinrauf & Geddes, 1976) which have sevenfold molecular symmetry and which had not yielded to other methods of crystal structure analysis. The rotation function (Rossmann & Blow, 1962) was designed to establish molecular symmetry axes and their orientation to the unit-cell axes. We therefore decided to survey reciprocal space in polar coordinates using the constant value of $\chi = 120°$ necessary to detect threefold rotational symmetry, and with φ and ψ varying from 0 to 180° in 10° increments. For this search, 1289 reflections between 12 and 3 Å resolutions were used. The stereographic projection of this rotation function is given in Fig. 1. Only one strong peak was produced, at $\psi = 120°$, $\varphi = 170°$. This peak was rather broad and a closer search with higher-resolution data did not resolve any details of the peak. Therefore, these results suggest that the two crystallographically independent prolinomycin molecules are very nearly parallel (within the accuracy of the rotation function; about 5°). With this knowledge we then calculated the Rb-phased electron-density map, so skewed as to be viewed down the threefold axis, and with the three thirds of each prolinomycin molecule averaged together. This map clearly showed the backbone atoms of one prolinomycin molecule and most of the second. A second electron-density synthesis based on the atoms now known revealed the rest of the atoms of the structure except for a few atoms of the solvent molecules. All atoms except for the methyl C of the toluene in the special position were obtained from a subsequent electron-density synthesis. The solvent molecules were surprisingly well ordered. There are one each of toluene, chloroform, and picrate in general positions. Another toluene molecule sits on a crystallographic center of symmetry. This toluene and the chloroform molecules are disordered. A packing diagram of the unit cell is shown in Fig. 5, mainly to illustrate the positions of the picrate anions and various solvent molecules. The atom Cl(315), part of the disordered chloroform, was omitted for the sake of clarity. Only the three Cl atoms of highest occupancy were included.

Several cycles of block-diagonal least-squares refinement with 116 nonhydrogen atoms brought the R index to 0·13 with the 4305 observed reflections and 0·18 for the 7240 measured reflections. The function minimized was $\sum w(|F_o| - |F_c|)^2$ where $w = 1·0$. Anisotropic thermal parameters were refined for the Rb and Cl atoms only. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1962). The final atomic coordinates and thermal parameters are given in Table 2.* The numbering scheme for

* Lists of structure factors, anisotropic thermal parameters for the Rb and Cl atoms and bond lengths and angles have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34979 (44 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

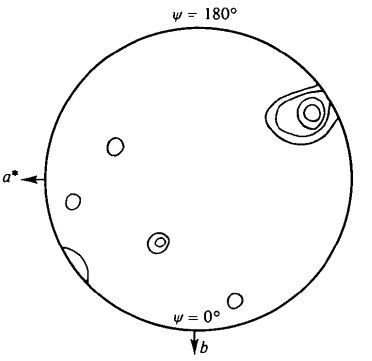

Fig. 1. The stereographic projection of the rotation function for prolinomycin. The contours are at arbitrary intervals.

Table 2. *Fractional atomic parameters* ($\times 10^4$) *and thermal parameters for the non-hydrogen atoms with e.s.d.'s in parentheses*

	x	y	z	U_{iso} (Å^2)		x	y	z	U_{iso} (Å^2)
N(1)	2583 (11)	5312 (11)	6766 (9)	5·4 (5)	O′(18)	773 (10)	1306 (10)	2715 (8)	7·1 (4)
C(2)	2798 (13)	4616 (13)	7001 (11)	5·0 (6)	C′(19)	1555 (16)	3284 (16)	2942 (14)	8·2 (8)
C(3)	3687 (13)	4875 (13)	7319 (12)	5·3 (6)	C′(20)	930 (17)	3854 (17)	3113 (15)	8·8 (8)
O(4)	3960 (9)	4324 (9)	7475 (8)	6·9 (4)	C′(21)	585 (16)	3871 (16)	2306 (14)	7·9 (8)
C(5)	2094 (16)	4407 (16)	7598 (14)	7·5 (7)	N′(22)	−354 (11)	1657 (11)	2324 (10)	5·9 (5)
C(6)	1955 (16)	5358 (16)	7976 (14)	7·6 (7)	C′(23)	−992 (14)	940 (14)	2578 (12)	5·5 (6)
C(7)	1962 (14)	5755 (14)	7324 (12)	6·2 (6)	C′(24)	−812 (14)	10 (14)	2092 (12)	6·0 (6)
N(8)	4153 (11)	5761 (11)	7494 (10)	5·8 (5)	O′(25)	−750 (10)	−121 (9)	1397 (8)	7·2 (4)
C(9)	5015 (14)	5964 (14)	7854 (12)	5·7 (6)	C′(26)	−1900 (14)	874 (14)	2363 (13)	6·4 (7)
C(10)	5580 (14)	5478 (14)	7233 (12)	5·7 (6)	C′(27)	−2092 (16)	1784 (16)	2871 (14)	7·8 (7)
O(11)	5602 (9)	5573 (9)	6576 (8)	6·3 (4)	C′(28)	−2581 (15)	89 (15)	2559 (13)	6·7 (7)
C(12)	5442 (14)	7043 (14)	8005 (12)	6·0 (6)	N′(29)	−740 (11)	−519 (11)	2453 (10)	5·8 (5)
C(13)	4912 (14)	7512 (14)	8627 (13)	6·7 (7)	C′(30)	−620 (15)	−1420 (15)	2020 (13)	6·5 (7)
C(14)	6399 (16)	7247 (16)	8321 (14)	7·9 (7)	C′(31)	285 (15)	−1336 (15)	1806 (13)	6·7 (7)
N(15)	5982 (10)	5036 (10)	7478 (9)	4·6 (4)	O′(32)	456 (9)	−2052 (9)	1424 (8)	7·0 (4)
C(16)	6562 (14)	4592 (14)	6926 (12)	5·5 (6)	C′(33)	−867 (17)	−1930 (17)	2635 (15)	9·0 (8)
C(17)	6027 (15)	3670 (15)	6413 (13)	7·1 (7)	C′(34)	−591 (20)	−1276 (20)	3383 (18)	12·2 (11)
O(18)	6464 (9)	3249 (9)	5913 (8)	6·6 (4)	C′(35)	−804 (17)	−429 (17)	3283 (15)	8·6 (8)
C(19)	7151 (16)	4420 (16)	7465 (14)	7·7 (8)	N′(36)	923 (11)	−588 (11)	2055 (9)	5·4 (5)
C(20)	6535 (15)	4211 (15)	8106 (13)	6·9 (7)	C′(37)	1823 (14)	−591 (14)	1816 (13)	6·3 (7)
C(21)	5984 (15)	4869 (15)	8228 (13)	6·6 (7)	C′(38)	1754 (15)	−776 (15)	949 (13)	7·0 (7)
N(22)	5188 (10)	3347 (10)	6480 (9)	5·2 (5)	O′(39)	1509 (9)	−385 (9)	600 (8)	6·9 (4)
C(23)	4729 (13)	2466 (13)	5934 (12)	5·4 (6)	C′(40)	2444 (15)	354 (15)	2185 (13)	6·8 (7)
C(24)	4810 (14)	2570 (14)	5164 (12)	6·0 (6)	C′(41)	3389 (16)	364 (16)	1857 (14)	7·7 (7)
O(25)	4662 (9)	3168 (9)	4966 (8)	6·0 (4)	C′(42)	2461 (17)	536 (17)	3052 (15)	9·1 (8)
C(26)	3745 (15)	2237 (15)	6172 (13)	7·2 (7)	C(201)	−2850 (19)	1828 (19)	−3124 (17)	10·8 (10)
C(27)	3685 (17)	2096 (17)	6954 (15)	8·8 (8)	C(202)	−2238 (16)	2518 (17)	−2668 (14)	8·4 (8)
C(28)	3252 (18)	1341 (18)	5539 (16)	10·0 (9)	C(203)	−1453 (18)	2725 (18)	−2943 (16)	10·0 (9)
N(29)	5132 (10)	1937 (10)	4610 (9)	4·9 (5)	C(204)	−1049 (25)	2343 (25)	−3733 (22)	15·4 (14)
C(30)	5196 (13)	1997 (13)	3819 (12)	5·3 (6)	C(205)	−1859 (18)	1588 (18)	−4079 (16)	9·9 (9)
C(31)	6023 (13)	2675 (13)	3686 (12)	5·4 (6)	C(206)	−2651 (25)	1361 (25)	−3834 (22)	15·3 (14)
O(32)	6108 (9)	2798 (8)	3057 (7)	5·6 (4)	O(207)	−3347 (17)	632 (17)	−4193 (15)	17·3 (10)
C(33)	5281 (16)	1010 (16)	3402 (14)	8·2 (8)	N(208)	−3672 (19)	1602 (19)	−2831 (17)	14·5 (10)
C(34)	5738 (16)	801 (16)	3990 (14)	7·7 (7)	O(209)	−4411 (12)	1223 (23)	−3223 (21)	23·6 (14)
C(35)	5336 (15)	1174 (15)	4759 (13)	6·6 (7)	O(210)	−3846 (19)	1976 (19)	−2161 (17)	19·3 (11)
N(36)	6620 (10)	3112 (10)	4265 (9)	4·8 (5)	N(211)	−775 (20)	3607 (20)	−2409 (17)	15·4 (11)
C(37)	7391 (13)	3770 (13)	4099 (11)	5·0 (6)	O(212)	−1079 (15)	3992 (15)	−1808 (14)	14·8 (8)
C(38)	7153 (14)	4466 (14)	3856 (12)	6·0 (6)	O(213)	−84 (20)	3824 (21)	−2725 (18)	21·2 (12)
O(39)	6631 (9)	4876 (9)	4259 (8)	6·7 (4)	N(214)	−1561 (27)	1075 (27)	−4898 (24)	22·3 (17)
C(40)	8012 (14)	4191 (14)	4810 (12)	5·8 (6)	O(215)	−2015 (23)	1018 (24)	−5373 (21)	24·4 (15)
C(41)	8806 (16)	4916 (16)	4710 (14)	7·9 (8)	O(216)	−1081 (23)	707 (24)	−4835 (21)	24·4 (15)
C(42)	8292 (17)	3492 (17)	5055 (15)	8·8 (8)	C(301)	4058 (34)	381 (35)	9585 (31)	23·0 (21)
N′(1)	−2144 (12)	1467 (12)	−521 (10)	7·0 (6)	C(302)	3302 (25)	770 (25)	9729 (22)	15·2 (14)
C′(2)	−2145 (15)	1663 (15)	323 (13)	6·9 (7)	C(303)	3300 (27)	1284 (28)	9441 (25)	17·3 (16)
C′(3)	−1271 (15)	2312 (15)	699 (13)	7·3 (7)	C(304)	3896 (24)	1684 (24)	8903 (22)	14·9 (13)
O′(4)	−1219 (10)	2470 (10)	1396 (9)	7·7 (5)	C(305)	4504 (30)	1287 (31)	8795 (27)	19·5 (18)
C′(5)	−2863 (18)	2162 (18)	474 (16)	9·6 (9)	C(306)	4668 (27)	628 (27)	9059 (24)	17·4 (15)
C′(6)	−2760 (18)	2659 (18)	−113 (16)	10·4 (9)	C(307)	2705 (26)	1819 (26)	9445 (23)	16·3 (15)
C′(7)	−2481 (17)	2021 (17)	−881 (15)	8·7 (8)	C(313)	1218 (30)	2118 (30)	4510 (27)	19·5 (18)
N′(8)	−619 (11)	2599 (11)	255 (9)	5·9 (5)	C(321)	4875 (24)	4170 (24)	−146 (22)	15·0 (14)
C′(9)	193 (14)	3163 (14)	663 (12)	5·8 (6)	C(322)	4316 (29)	4509 (30)	−360 (27)	19·2 (18)
C′(10)	505 (15)	2673 (15)	1074 (14)	7·5 (7)	C(323)	4078 (25)	5332 (26)	−318 (23)	15·8 (14)
O′(11)	658 (9)	1918 (9)	715 (8)	6·9 (4)	Rb†	5000	5000	5000	7·42
C′(12)	833 (16)	3457 (15)	64 (14)	7·7 (7)	Rb′	0	0	0	7·50
C′(13)	1755 (18)	3949 (18)	443 (16)	9·5 (9)	Cl(311)‡	1085 (9)	1113 (8)	4678 (7)	11·52
C′(14)	566 (18)	4071 (18)	−325 (16)	9·9 (9)	Cl(312)	266 (11)	2454 (9)	4569 (10)	19·74
N′(15)	784 (11)	2970 (11)	1810 (10)	6·1 (5)	Cl(314)	2234 (17)	2909 (15)	4856 (15)	26·31
C′(16)	1094 (14)	2534 (14)	2199 (12)	6·1 (6)	Cl(315)	1471 (31)	2855 (21)	5593 (25)	26·51
C′(17)	523 (14)	1782 (14)	2426 (12)	5·7 (6)					

† The temperature factors of the rubidium and chlorine atoms given here are equivalent isotropic values calculated from the anisotropic parameters using the formula $U_{eq} = \frac{1}{3} \sum_i \sum_j (U_{ij} a_i^* a_j^* \bar{a}_i \bar{a}_j$. The anisotropic values have been deposited.

‡ The occupancy factors for the four chlorine atoms are 0·62 (2), 0·71 (3), 0·59 (3) and 0·37 (3) respectively.

Table 3. *Rubidium coordination*

		∠C=O···Rb
Molecule (I)		
Rb···O(11)	2·888 (14) Å	152·1 (11)°
Rb···O(25)	2·861 (12)	152·6 (12)
Rb···O(39)	2·928 (15)	155·1 (12)
Molecule (II)		
Rb′···O′(11)	2·917 (13)	149·0 (13)
Rb′···O′(25)	2·798 (14)	158·3 (13)
Rb′···O′(39)	3·014 (17)	148·1 (14)

Table 4. *Intramolecular hydrogen-bond lengths and angles*

		∠C=O···N
N(1)···O(32)	3·136 (19) Å	107·8 (10)°
N(15)···O(4)	3·134 (21)	106·0 (12)
N(29)···O(18)	3·138 (19)	106·2 (13)
N′(1)···O′(32)	3·160 (23)	106·4 (13)
N′(15)···O′(4)	3·174 (24)	105·7 (14)
N′(20)···O′(18)	3·191 (20)	107·1 (12)

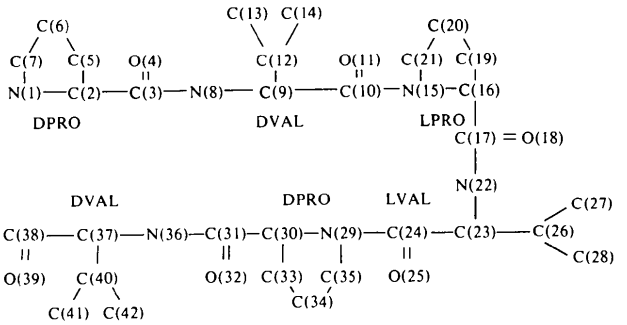

Fig. 2. The numbering scheme for the prolinomycin molecules. The numbers shown refer to the asymmetric half of molecule (I). The other half of the molecule is related by the crystallographic center of symmetry. The numbering for molecule (II) is identical to that of molecule (I), except that the numbers are primed.

prolinomycin, the picrate anion and the solvent molecules is given in Figs. 2 and 3. The coordination distances of the rubidium ions to the oxygen ligands are shown in Table 3 and the hydrogen-bond lengths in Table 4.

The bond lengths and angles for the prolinomycin molecules do not deviate (within experimental error) from standard values. The picrate molecules show reasonably good agreement with the crystal structure of potassium picrate (Maartman-Moe, 1969). However, the solvent molecules are disordered in varying degrees and did not refine to good atomic parameters. Population parameters were refined in the case of the Cl atoms of the chloroform molecule and their total contribution (2·3 atoms) indicates that these solvent positions are far from full occupation. Cl atoms appear in four approximately tetrahedral positions around the central C atom, evidently reflecting a very non-specific binding of the chloroform molecule.

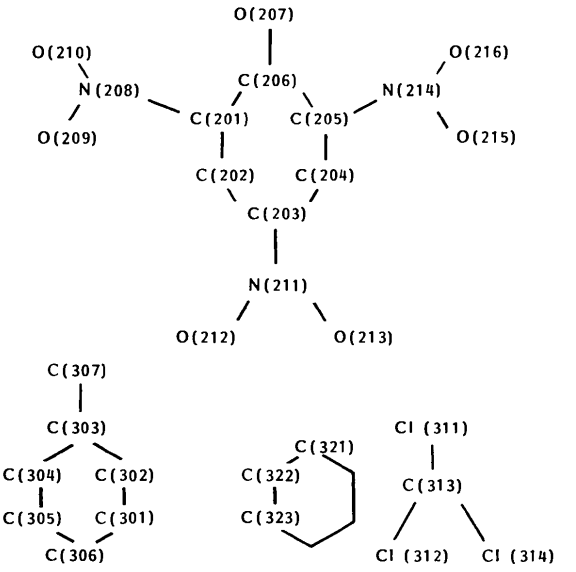

Fig. 3. The numbering scheme for the picrate anion, the toluene molecules in general and special positions and the chloroform molecule.

Discussion

A stereodrawing of the two independent molecules of prolinomycin is given in Fig. 4, and a packing diagram in Fig. 5. The rubidium ions are found at 0,0,0 and $\frac{1}{2},\frac{1}{2},\frac{1}{2}$. The two different prolinomycin molecules sit on different centers of symmetry. Each complex has a center of symmetry which results in two different half complexes in the crystallographic asymmetric unit. A

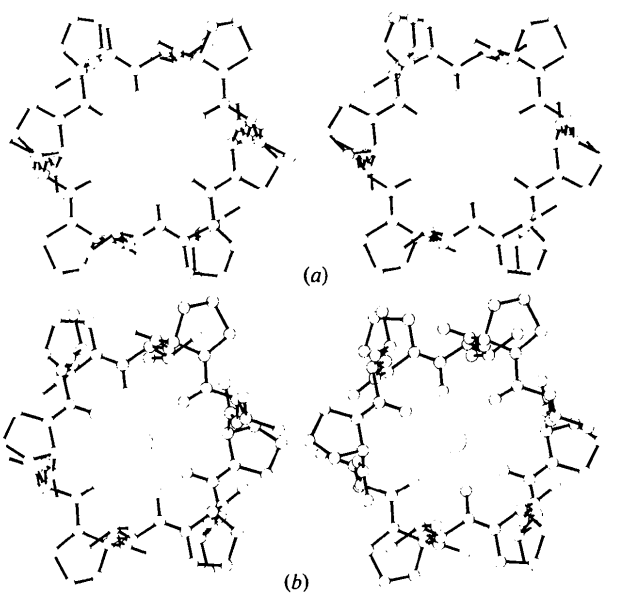

Fig. 4. A stereoview of (*a*) molecule (I) and (*b*) molecule (II) of prolinomycin viewed approximately down the molecular three-fold axis.

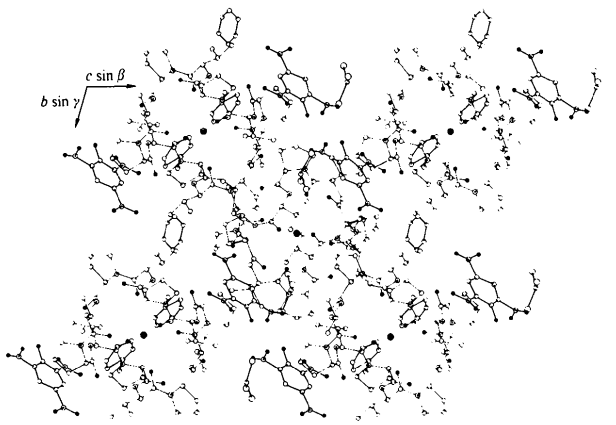

Fig. 5. A packing diagram of the unit cell, included to show the positions of the anions and solvent molecules. The latter are drawn with darker bonds than those of the prolinomycin molecules. ○ Carbon, • oxygen, ⊙ nitrogen, ● rubidium and O chlorine.

picrate anion occupies a general position and, with the identical picrate related by the center of symmetry, provides the electrical neutrality for the crystal.

As may be seen qualitatively from Fig. 4, the conformations of the two independent prolinomycin complexes are essentially the same. This conformation is also very similar to that found for the valinomycin potassium tetrachloroaurate(III) complex by Pinkerton, Steinrauf & Dawkins (1969). However, the prolinomycin is much closer to threefold molecular symmetry. This threefold symmetry is also followed by the side chains. The isopropyl groups of the valyl residues are in the staggered conformation of ±60 and 180°. Molecular-energy calculations (Hamilton, Sabesan, Gisin & Steinrauf, 1978) indicated that prolinomycin may form a slightly more stable complex with Rb than does valinomycin.

Prolinomycin, like valinomycin, is a very rigid cage. Due to hydrogen bonding the circumference of this cage is particularly inflexible, and, since prolinomycin cannot adapt to a larger or smaller ion by a change in circumference, it must compromise by changing from a circle to an ellipse to preserve the center of symmetry. The prolinomycin molecule adapts to the rubidium ion, which is slightly too small, by providing a centrosymmetric pair of short bonds, a pair of medium bonds, and a pair of longer bonds to the rubidium ion. This is in contrast to providing six medium-length bonds in valinomycin. Of course, in solution the requirement of a center of symmetry is no longer necessary and other adaptations may be used by the molecule. In the crystal structure of valinomycin rubidium tetrachloroaurate(III), also under investigation in this laboratory, the rubidium ion is significantly closer to the D-valyl residues than to the L-valyl residues.

The two prolinomycin molecules are very similar. The averages of the Rb···O bonds are 2·892 and 2·910 Å for molecule (I) and molecule (II) respectively. This represents a difference of about one standard deviation (0·015 Å).* These Rb···O bond

* All standard deviations for averaged bond lengths and angles in this discussion were obtained by taking the mean of the individual standard deviations produced by the least-squares refinement.

Table 5. *Conformation angles (°) of the prolinomycin molecules compared with those of the complexed and uncomplexed valinomycin*

The residues related by noncrystallographic threefold symmetry (*e.g.* *R*1, *R*5, *R*9) are grouped together for convenience. The convention for the conformation angles is that proposed by the IUPAC–IUB Commission on Biochemical Nomenclature (1970).

			D-Pro*			D-Val			L-Pro*			L-Val			
Angle	Antibiotic	Ions	*R*1	*R*5	*R*9	*R*2	*R*6	*R*10	*R*3	*R*7	*R*11	*R*4	*R*8	*R*12	Reference
φ	Prolinomycin	Rb picrate	76	83	84	66	58	63	−84	−76	−83	−63	−66	−58	Present work†
		Rb picrate	83	80	72	60	55	61	−72	−83	−80	−61	−60	−55	Present work
	Valinomycin	$Rb[AuCl_4]$	91	79	70	49	56	62	−85	−82	−91	−67	−60	−62	(*a*)
		KI_5	79	86	79	56	58	59	−66	−73	−76	−58	−60	−57	(*b*)
		Uncomplexed	96	146	82	63	60	108	−74	−98	−164	−63	−108	−67	(*c*)
		Uncomplexed	99	150	77	68	63	104	−75	−96	−162	−68	−110	−65	(*c*)
		Uncomplexed	98	147	81	67	54	105	−71	−100	−165	−67	−110	−71	(*d*)
		Uncomplexed	94	147	78	67	64	108	−71	−97	−166	−66	−108	−59	(*d*)
		Uncomplexed	98	145	80	65	65	106	−77	−98	−160	−64	−102	−63	(*d*)
ψ	Prolinomycin	Rb picrate	12	3	1	−132	−133	−128	−1	−12	−3	128	132	133	Present work
		Rb picrate	−2	3	10	−132	−133	−131	−10	2	−3	131	132	133	Present work
	Valinomycin	$Rb[AuCl_4]$	4	11	11	−130	−137	−132	−9	−9	−2	131	137	142	(*a*)
		KI_5	3	−5	8	−129	−131	−133	−25	−16	−12	131	133	133	(*b*)
		Uncomplexed	−3	−11	3	−134	−135	−69	−6	14	23	129	78	130	(*c*)
		Uncomplexed	−8	−12	8	−134	−134	−71	−11	6	27	130	78	132	(*c*)
		Uncomplexed	−6	−7	3	−136	−133	−68	−11	13	31	130	80	132	(*d*)
		Uncomplexed	−5	−10	7	−136	−134	−68	−9	7	22	129	78	132	(*d*)
		Uncomplexed	−4	−8	2	−134	−135	−71	−7	10	21	128	74	131	(*d*)

References: (*a*) Steinrauf (unpublished results); (*b*) Neupert-Laves & Dobler (1975); (*c*) Karle (1975); (*d*) Smith *et al.* (1975).

* In valinomycin, D-Pro residues are replaced by D-hydroxyisovaleric acids and L-Pro are replaced by L-lactic acids.

† The standard deviation in the conformation angles (φ, ψ) for prolinomycin varies from 1·5 to 2·6° (average 2·1°).

lengths are, however, slightly longer than the average value of 2·833 Å, standard deviation 0·011 Å, found by us for valinomycin rubidium chloride. The C–O···Rb bond angles, averaged for the two individual prolinomycin complexes, give 153·3 and 152·0°, which are within one standard deviation (1·2°). The corresponding values from the valinomycin rubidium tetrachloroaurate(III) complex average 158·3°, standard deviation 1·1°. The average of the six independent hydrogen bonds is 3·16 Å, which is about 0·1 Å longer than those found in valinomycin structures, and is in agreement with the concept that prolinomycin is a slightly larger cage.

The constraints imposed by the substitution of the prolyl residues for the α-hydroxy residues have not resulted in any significant changes for the complex. However, if we extrapolate to the conformation found for the uncomplexed valinomycin, we find a serious problem with prolinomycin. As may be seen in Table 5, uncomplexed valinomycin requires a φ angle of 145 to 150° for one of its D-isovaleryl residues and −160 to −166° for one of its L-lactyl residues. Such values are not possible for prolyl residues. Typical values from small peptides containing L-proline are found to be from −50 to −68°, as given by Balasubramanian, Lakshminarayan, Sabesan, Tegoni, Venkatesan & Ramachandran (1971). We therefore suggest that the most important low-energy conformations are not available to prolinomycin in the uncomplexed state. Since the usual methods for measuring membrane transport require the neutral carrier to be in the uncomplexed form during the return half-cycle of transport, prolinomycin would be expected to be a poorer carrier than is valinomycin, in spite of being as good (or better) a binding agent for monovalent cations. Furthermore, we also suggest that the rate at which prolinomycin could capture or release a cation should be slower than that of valinomycin, again because of the rotational restrictions of the prolyl residues. These considerations are in agreement with the extensive studies on the transport properties of valinomycin and prolinomycin by Benz *et al.* (1976).

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