quinolinate and nicotinamide formation can thus be precisely controlled by modifications of the enzymes involved in the biosynthesis of **2** and in the metabolism of **2** by another pathway. Thus, although it is unusual to have a nonenzymic step compete with an enzymic one at a branchpoint in an important metabolic pathway, it is perhaps not too surprising since effective control, even of the products of the nonenzymic reaction, can still be achieved.

The Hydrated Potassium Complex of the Ionophore Monensin A

Walter Pangborn,* William Duax, and David Langs

Contribution from the Medical Foundation of Buffalo, Inc., Buffalo, New York 14203.
Received July 7, 1986

Abstract: The crystal structure of the potassium monensin A dihydrate has been determined (orthohombic, space group $P2_12_12_1$, a = 16.485 (2) Å, b = 19.189 (2) Å, c = 12.661 (1) Å, Z = 4). Examination of the conformation of the K^+ complex compared with that of the previously reported structures of several complexes, particularly the Na^+ complexes, reveals several features which correlate with the observed selectivity of monensin for Na^+ over K^+. Coordination of the K^+ ion involves changes in the conformation of a spiro-fused ring, the conformation of which is highly conserved in the structures of several Na^+ complexes. Preliminary molecular mechanics calculations are consistent with the conclusion that the coordination of the K^+ ion involves a distortion of the spiro-fused ring from its low-energy conformation. Furthermore, the coordination of the K^+ ion is less uniform than that observed in the Na^+ complexes.

The antibiotic monensin A (Figure 1) is a biologically active compound produced by a strain of *Streptomyces cinnamonensis*. A member of the family of monocarboxylic acid, polycyclic, polyether antibiotics, it induces monovalent cation permeability, exhibits selectivity for Na^+ over K^+, and has a greater stability constant with Na^+ than K^+.[1]

The selectivity of ionophores must be largely influenced by the relative binding energies of the various cations. Among the factors influencing this would be cation size, the distribution of ligands, and the conformational flexibility of the ionophore. However, the relative importance of the various parameters affecting selectivity is disputed. In order to attempt to relate structural features to the selectivity of ionophores, the structures of the free acid should be compared with those of the complexes of at least two cations with different selectivities.

Monensin is one of the ionophores for which the most crystallographic data are available. For monensin A, the structures of the free acid,[2] the hydrated and anhydrous forms of Na^+ complex,[3] the NaBr complex,[4] and the hydrated Ag^+ complex[5] have been determined. Also, the monohydrate of the Na^+ complex of monensin B,[6] and the Li^+ and Ag^+ salts of monensin B,[7] which differs from monensin A by the replacement of an ethyl group by a methyl group, have been determined. The crystal structure of the dihydrate of the K^+ complex of monensin A reported here provides the data required to compare the structures of the complexes of monensin A with cations with which it exhibits selectivity.

Experimental Section

Single crystals of the dihydrate of the potassium complex of monensin A, $C_{36}H_{61}O_{11}K\cdot 2H_2O$, were grown from an ethanol/water solution. These crystals are isomorphous with previously reported dihydrate sodium and silver complexes of monensin A ($P2_12_12_1$, a = 16.485 (2) Å, b = 19.189 (2) Å, c = 12.661 (1) Å, V = 4005 Å^3, Z = 4, ρ_c = 1.24 g cm^{-3}). A single crystal (0.4 × 0.8 × 0.8 mm) was mounted and a total of 4610 independent data ($\sin\theta_{max}/\lambda$ = 0.61) were collected on an Enraf-Nonius CAD-4 diffractometer with use of nickel-filtered copper radiation. Intensities were corrected for Lorentz and polarization factors but not for extinction or absorption (μ(Cu Kα) = 16.5 cm^{-1}). Of the 4610 measured data, 4499 were considered observed on the basis of $I > 2\sigma I$. The variance of each F was calculated according to the method of Stout and Jensen[8] with an instability correction of 0.04. Unobserved data were given zero weight.

The starting coordinates were those reported for the isomorphous hydrated sodium complex.[3] The structure was refined by full matrix least squares, minimizing $\sum w(|F_o| - |F_c|)^2$, treating all non-hydrogen atoms anisotropically. In the final cycles of refinement, hydrogen atoms, except for those of hydroxyl groups, were included at calculated positions. The refinement converged to a final residual of 0.046 and a weighted residual of 0.068. Positional parameters appear in Table I.

Results and Discussion

Superficially, all the cation complexes of monensin A are similar. In the K^+ complex, as in other complexes, two intramolecular hydrogen bonds involving the carboxyl oxygens and two hydroxy groups at the opposite end of the molecule are found. These head-to-tail hydrogen bonds (O(1)–O(11) = 2.51 Å and O(2)–O(10) = 2.62 Å), common in this class of antibiotics, produce a pseudocyclic conformation which serves to form the cavity in which the cation is coordinated. This intramolecular hydrogen bonding and cation coordination are illustrated in Figure 2. Bond lengths and angles are illustrated in Figure 3. The crystal packing and intermolecular hydrogen bonding found here are similar to that reported in the hydrated Na^+ complex.

All hydrogen bond distances are listed in Table II. In addition to a hydrogen bond between the two independent water molecules, each water is involved in two hydrogen bonds to the ionophore. As in the hydrated Na complex, there is an ionophore water layer perpendicular to the y axis. Adjacent layers are related by the screw axis parallel to y. There are no hydrogen bonds between layers.

(1) Dobler, M. *Ionophores and Their Structures*; Wiley-Interscience: New York, 1981; pp 16, 20, 77–83.
(2) Lutz, W. K.; Winkler, F. K.; Dunitz, J. D. *Helv. Chim. Acta* **1971**, *54*, 1103.
(3) Duax, W. L.; Smith, G. D.; Strong, P. D. *J. Am. Chem. Soc.* **1980**, *102*, 6725.
(4) Ward, D. L.; Wei, K. T.; Hoogerheide, J. C.; Popov, A. I. *Acta Crystallogr., Sect. B* **1978**, *B34*, 110.
(5) Pinkerton, M.; Steinrauf, L. K. *J. Mol. Biol.* **1970**, *49*, 533.
(6) Barrans, P. Y.; Alleume, M.; Jeminet, G. *Acta Crystallogr., Sect B* **1982**, *B38*, 1144.
(7) Walba, D. M.; Hermsmeir, M.; Haltiwanger, R. C.; Noordik, J. H. *J. Org. Chem.* **1986**, *51*, 245.
(8) Stout, G. H.; Jensen, L. H. *X-Ray Structure Determinations*; Macmillan: New York, 1968; p 457.

0002-7863/87/1509-2163$01.50/0

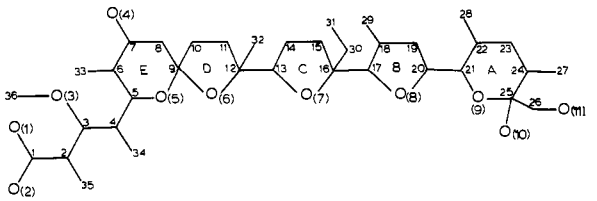

Figure 1. Chemical structure, numbering scheme, and ring indentification of monensin A.

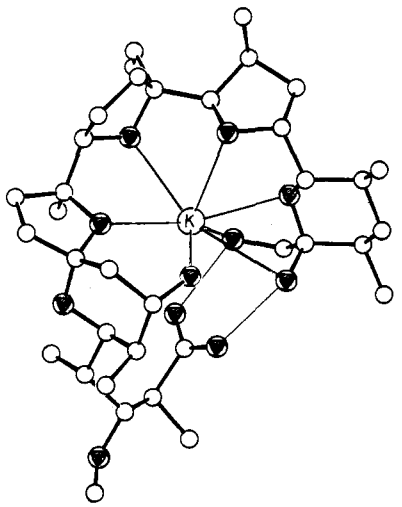

Figure 2. An illustration of the intramolecular hydrogen bonding and the cation coordination in the potassium monensin A complex.

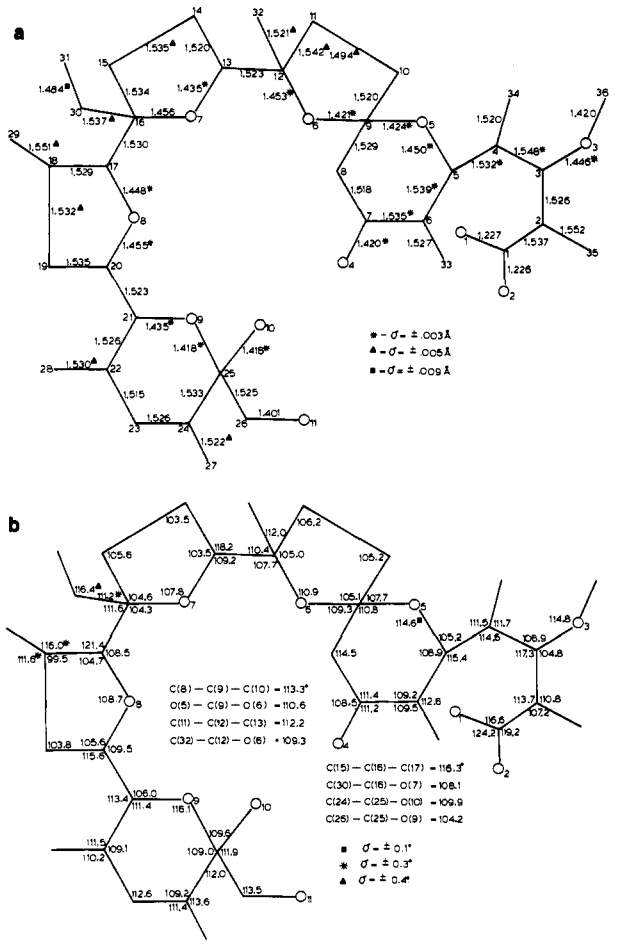

Figure 3. (a) Bond lengths and (b) valence angles for the dihydrate crystal of the potassium complex of monensin A. The standard deviation for all bond lengths not noted in the figure is ±0.004 Å, and for all bond angles not noted it is ±0.2 Å.

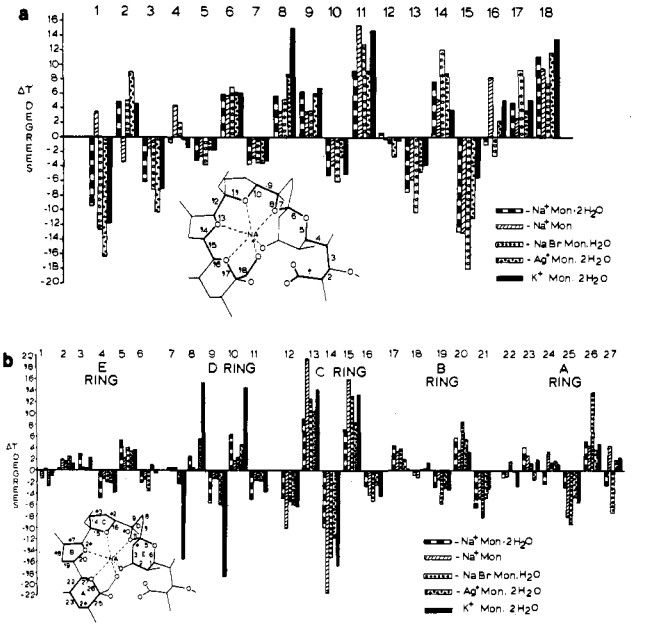

Figure 4. (a) The differences in the backbone torsion angles of the metal ion complexes of monensin A from those of the free acid. (b) The differences in the intraring torsion angles of the metal complexes of monensin A from those of the free acid.

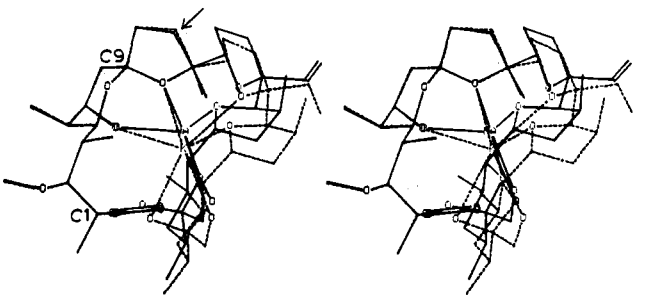

Figure 5. Stereo diagram illustrating the expansion of the coordination sphere in the K^+ complex (dashed) compared to the Na^+ complex (solid). This expansion is achieved by a pseudorotation in the D ring (arrow).

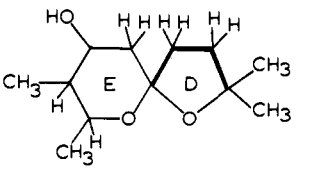

Figure 6. Monensin fragment used in molecular mechanics calculation to compare the relative stability of the D-ring conformation observed in the Na^+ and K^+ complexes of monensin A.

Figure 4a illustrates the deviation of the backbone torsion angles of the various monensin A complexes from those of the free acid; Figure 4b illustrates the deviations of the ring torsion angles of the complexes from those of the free acid. The conformations of all the complexes except the K^+ complex differ from the free acid in a very similar fashion. The K^+ complex, however, exhibits significant differences from the other complexes in ring D. This is part of the spiro-fused ring which one would expect to be the least flexible portion of the molecule and the conformation of which is highly conserved in all except the K^+ complex. These torsion angle changes in ring D result in a pseudorotation from one envelope form to another, acting as a hinge to expand the coordination sphere relative to that found in the Na^+ complex to accommodate the larger K^+ ion. This expansion is illustrated in Figure 5.

Molecular mechanics calculations[9] were performed on the 36-atom fragment shown in Figure 6 which includes the spiro-fused

(9) Ailinger, N. L.; Yuh, Y. H. *QCPE* **1980**, No. 395.

Table I. Atomic Coordinates of K Monensin A

atom	*x/a*	*y/b*	*z/c*
K	0.37553 (4)	0.33988 (3)	−0.16268 (5)
C(1)	0.2764 (2)	0.2089 (1)	−0.3390 (2)
C(2)	0.2185 (2)	0.1991 (1)	−0.4331 (2)
C(3)	0.1497 (2)	0.2522 (1)	−0.4365 (2)
C(4)	0.1733 (1)	0.3299 (1)	−0.4481 (2)
C(5)	0.1960 (1)	0.3661 (1)	−0.3444 (2)
C(6)	0.1300 (2)	0.3653 (1)	−0.2585 (2)
C(7)	0.1600 (2)	0.4065 (1)	−0.1622 (2)
C(8)	0.1898 (2)	0.4783 (1)	−0.1936 (2)
C(9)	0.2458 (2)	0.4792 (1)	−0.2899 (2)
C(10)	0.2605 (2)	0.5519 (1)	−0.3334 (3)
C(11)	0.3465 (2)	0.5526 (2)	−0.3705 (3)
C(12)	0.3868 (2)	0.4873 (1)	−0.3226 (2)
C(13)	0.4564 (2)	0.5058 (1)	−0.2485 (2)
C(14)	0.4375 (2)	0.5493 (1)	−0.1513 (3)
C(15)	0.5049 (3)	0.5292 (1)	−0.0735 (3)
C(16)	0.5425 (2)	0.4622 (1)	−0.1178 (3)
C(17)	0.5410 (2)	0.3985 (1)	−0.0450 (2)
C(18)	0.5997 (2)	0.3918 (2)	0.0480 (3)
C(19)	0.5571 (2)	0.3337 (2)	0.1099 (3)
C(20)	0.4665 (2)	0.3499 (1)	0.0959 (2)
C(21)	0.4114 (2)	0.2868 (1)	0.0835 (2)
C(22)	0.4111 (2)	0.2391 (2)	0.1800 (2)
C(23)	0.3658 (2)	0.1727 (2)	0.1533 (2)
C(24)	0.3978 (2)	0.1379 (1)	0.0532 (3)
C(25)	0.3951 (1)	0.1904 (1)	−0.0380 (2)
C(26)	0.4390 (2)	0.1633 (2)	−0.1358 (2)
C(27)	0.3528 (3)	0.0703 (2)	0.0300 (4)
C(28)	0.3721 (3)	0.2746 (3)	0.2755 (3)
C(29)	0.6113 (3)	0.4581 (2)	0.1163 (4)
C(30)	0.6279 (2)	0.4756 (2)	−0.1620 (4)
C(31)	0.6609 (3)	0.4220 (4)	−0.2347 (5)
C(32)	0.4155 (2)	0.4368 (2)	−0.4073 (3)
C(33)	0.0497 (2)	0.3959 (2)	−0.2968 (3)
C(34)	0.2383 (2)	0.3404 (2)	−0.5319 (2)
C(35)	0.1845 (3)	0.1238 (2)	−0.4263 (3)
C(36)	0.0182 (2)	0.2443 (3)	−0.5195 (3)
O(1)	0.3483 (2)	0.2175 (3)	−0.3601 (3)
O(2)	0.2489 (2)	0.2080 (2)	−0.2490 (2)
O(3)	0.1031 (1)	0.2340 (1)	−0.5292 (1)
O(4)	0.2242 (1)	0.3710 (1)	−0.1102 (1)
O(5)	0.2131 (1)	0.43784 (8)	−0.3730 (1)
O(6)	0.3237 (1)	0.45434 (9)	−0.2600 (1)
O(7)	0.4899 (1)	0.44288 (9)	−0.2054 (2)
O(8)	0.4612 (1)	0.3932 (1)	0.0019 (2)
O(9)	0.4393 (1)	0.2508 (1)	−0.0089 (1)
O(10)	0.3135 (1)	0.2089 (1)	−0.0603 (1)
O(11)	0.4506 (1)	0.2143 (1)	−0.2136 (2)
O(2W)	0.1826 (2)	0.2762 (1)	0.0412 (2)
O(1W)	0.6143 (2)	0.2403 (2)	−0.2406 (2)

ring and adjacent atoms. The calculation indicates that the conformations observed in the free acid and all except the K^+ complex are essentially indistinguishable from the minimum energy conformation. Fixing the value of the torsion angle shown in bold in Figure 6 to its observed value for both the Na^+ and K^+ complex and again minimizing the energy of these fragments yields an energy for the Na^+-like fragment 2.3 kcal/mol lower than that for the K^+ ion form. These results are qualitative only, but they are consistent with the conclusion that the introduction of the larger K^+ ion into the coordination site of monensin A necessitates the distortion of a constrained portion of the molecule from its low-energy conformation.

Table II. Hydrogen Bond Distances (Å)

O(1)–O(11)	2.51[a]	O(4)–O(2W)	2.73
O(2)–O(10)	2.62[a]	O(11)–O(1W)	2.76
		O(10)–O(2W)	2.82
O(3)–O(1W)	2.96	O(1W)–O(2W)	2.78

[a] Intramolecular.

Table III. Potassium Ion Coordination

	distance, Å		angle, deg
O(4)–K	2.650	O(4)–K–O(6)	68.1
		O(4)–K–O(7)	121.7
		O(4)–K–O(8)	102.8
		O(4)–K–O(9)	108.5
		O(4)–K–O(11)	132.2
O(6)–K	2.660	O(6)–K–O(7)	62.7
		O(6)–K–O(8)	102.2
		O(6)–K–O(9)	161.5
		O(6)–K–O(11)	138.6
O(7)–K	2.785	O(7)–K–O(8)	62.0
		O(7)–K–O(9)	108.3
		O(7)–K–O(11)	105.6
O(8)–K	2.717	O(8)–K–O(9)	60.1
		O(8)–K–O(11)	105.8
O(9)–K	2.796	O(9)–K–O(11)	57.7
O(11)–K	2.786		

The coordination distances and angles of the K^+ ion appear in Table III. The K^+ complex is found to have an even more severely distorted distribution of coordinating ligands than is found in the Na^+ complex. Although monensin can expand to achieve coordination of the K^+ ion, the constraints imposed by the molecular skeleton result in the displacement of most of the oxygens toward one side of the K^+ ion and therefore a non-uniform distribution of ligands around the ion.

We conclude that the selectivity exhibited by monensin A for Na^+ over K^+ is due to the energetic cost of distorting a constrained portion of the molecule in order to accommodate the larger K^+ ion and the less favorable distribution of ligands about the ion.

Acknowledgment. We thank Lilly Research Laboratories for supplying the monensin A used in this study. This investigation was supported by Grant GM32812 awarded by the National Institutes of General Medical Sciences, DHHS.

Supplementary Material Available: Tables of thermal parameters and torsion angles (2 pages); listing of structure factors (21 pages). Ordering information is given on any current masthead page.