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- (28) For example, the ion resulting from loss of 15 mass units is formulated as the methyl isopropyl ketone molecular ion.²⁷ This ion is expected and observed to fragment further by loss of methyl and isopropyl radicals giving m/z 71 and 43.
- (29) A referee has emphasized that the MIKES methodology stands in contrast to solution chemistry in that its selectivity might cause attention to be focused on minor side reactions.

Structure of a Hydrated Sodium-Lasalocid A (X-537A) Dimer: an Intermediate in Complex Formation

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Abstract: A sodium-lasalocid A-water (2:2:2) complex, crystallized from 95% ethanol, appears to be an intermediate in the monomer to dimer transition that accompanies ion capture and transport. The complex crystallizes in the monoclinic space group $P2_1$ with cell dimensions $a = 12.148$ (2) Å, $b = 27.589$ (3) Å, $c = 11.802$ (3) Å, $\beta = 110.25$ (1)°, and $Z = 2$. The structure consists of two sodium ions and two water molecules enclosed by two lasalocid A ions. Six of the seven oxygens coordinated to one sodium ion are contributed by both lasalocid A ions; a water molecule provides the seventh site. The other sodium ion is coordinated to four oxygens of a single lasalocid A ion and both water molecules.

In the past several years lasalocid A (X-537A) (Figure 1) has been the subject of a great many biochemical investigations because of its ability to transport ions across natural and synthetic membranes.¹⁻³ It has also been extensively studied by X-ray crystallographic techniques in order to determine the structure of this ionophore complexed to a variety of monovalent and divalent ions. Results of the crystallographic studies have shown that the free acid and complexed forms can exist as both monomers and dimers. Dimeric structures have been observed for the 1:2 barium-lasalocid A,⁴ the 1:2 water-lasalocid A,⁵ and two distinctly different 2:2 sodium-lasalocid A⁶ complexes. Differences in the torsion angles in the backbone of the 12 independent X-537A molecules or anions present as monomers or dimers are not more than 26°.

The structures of all of the dimeric forms consist of a "sandwich"-type complex in which the complexed molecule or ion resides in a cavity between the two ionophores. Two classes of dimers have been observed, those in which there is head to tail association of the molecules forming the dimer (observed in the barium and silver salts and one form of the sodium complex) and head to head dimers (observed in the water complex and the second form of the sodium complex). The surfaces of both types of dimeric complexes are composed primarily of hydrophobic groups and it seems clear that one or both of these dimeric forms are responsible for transport through nonpolar media.

Observed monomeric species, a free acid form and a sodium ion complex,⁷ contain a methanol molecule which forms hydrogen bonds to the ionophore; in addition, the oxygen of the methanol molecule fills out the coordination sphere of the sodium ion.

It has been suggested that metal uptake and release in polar environments involve monomeric forms, while transport in nonpolar media takes place by means of a lasalocid dimer. In addition, the results of NMR studies in nonpolar solvents have indicated the presence of a dimeric structure similar to but not

identical with that observed for the sodium ion complexes in the solid state.⁶ We report here the crystal structure of a dimeric form of this ionophore complexed to both water and sodium ion.

Experimental Section

Single crystals of the dimeric 2:2:2 sodium-lasalocid A-water complex were grown from 95% ethanol. Unit cell data are given in Table I. The intensities of 3896 independent reflections ($\sin \theta_{\text{max}}/\lambda = 0.497 \text{ \AA}^{-1}$) were measured on an Enraf-Nonius CAD-4 diffractometer using Ni-filtered $\text{Cu K}\alpha$ radiation. No significant changes were observed in the intensities of two standard reflections which were measured after every 96 intensities were recorded. Intensities were corrected for Lorentz and polarization (Lp) factors but not for extinction or absorption. Real and imaginary dispersion corrections were applied to the atomic scattering factors.⁸ On the basis of a $2\sigma(I)$ test, 2942 data were considered observed. The variance of each F was calculated according to the method of Stout and Jensen⁹ [$\sigma^2(F) = k/4(Lp)I[\sigma^2(I) + (0.06I)^2]$; $w(F) = 1/\sigma^2(F)$]. Unobserved data were given zero weight and not included in the refinement.

The structure was solved through the use of the direct methods program QTAN¹⁰ and refined by full-matrix least squares, treating the vibration of carbon atoms isotropically and that of sodium and oxygen anisotropically, to a residual of 0.078 ($R = \sum ||F_o| - |F_c|| / \sum |F_o|$) for the observed data and 0.105 for all data.¹¹ The weighted residual ($R_w = [\sum w(|F_o| - |F_c|)^2 / \sum wF_o^2]^{1/2}$) was 0.106. Hydrogen atom contributions were included in the final two cycles of least-squares refinement by calculating their positions on the basis of the heavy-atom positions at the end of each cycle; methyl groups were assumed to have a staggered conformation. No contribution was included for the hydrogen of the hydroxy groups or the methyl attached to the benzoic acid group since these positions cannot be calculated unambiguously.

Discussion

The overall structure of the dimer, illustrated in Figure 2, consists of two sodium ions and two water molecules enclosed in a cavity constructed from the two lasalocid A ions. While

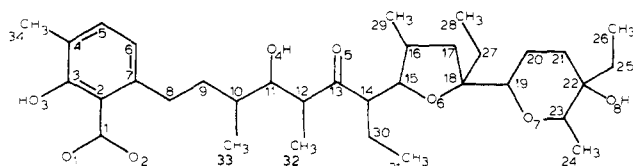


Figure 1. Primary structure of lasalocid A and the numbering system.¹

Table I. Unit Cell Data for $(\text{Na} \cdot \text{C}_{34}\text{H}_{53}\text{O}_8 \cdot \text{H}_2\text{O})_2$

$a = 12.148 (2) \text{ \AA}$	
$b = 27.589 (3) \text{ \AA}$	$\beta = 110.25 (1)^\circ$
$c = 11.802 (3) \text{ \AA}$	
$Z = 2$	space group $P2_1$
$d_c = 1.13 \text{ g cm}^{-3}$	$\mu(\text{Cu K}\alpha) = 7.61 \text{ cm}^{-1}$

a comparison of individual backbone torsion angles (Table II) of the two crystallographically independent lasalocid A ions reveals small differences (five torsion angles have differences equal to or greater than 10° with a maximum of 15°), a significant change in overall conformation does exist and is illustrated in Figure 3. This change apparently results from small differences in torsion angles acting in a concerted fashion to produce an overall modification in the conformation. Bond distances and angles within each lasalocid A ion are unexceptional.

The results of a least-squares molecular fit¹² in which the two ions of this study and the ion of the lasalocid-amine complex are compared to each other pairwise show that the conformation of each of the two independent lasalocid A ions is more nearly like its mate than that observed for the lasalocid-amine complex.¹³ These results are shown in Figure 3 and clearly illustrate the differences in conformation of the C(8) through C(14) region and, at the same time, the rigidity of the hydroxy end of the molecule; torsion angles of the monomeric amine complex are listed in Table II. Again, this observed difference in conformation cannot be ascribed to one or two torsion angles but rather is the result of the concerted action of many small changes in individual torsion angles.

Each lasalocid A ion possesses the intraionic hydrogen bond, O(1)-O(3), observed in all structural studies of this ionophore thus far. In all previous lasalocid crystal structure determinations, head to tail intraionic hydrogen bonding is found between hydroxy O(8) and one or both of the carboxylate oxygens O(1) or O(2). This is potentially true in only one of the lasalocid ions of this study (ion II of Figure 4). In this case the three atoms O(2), O(4), and O(8) form a nearly equilateral triangle with distances ranging from 2.85 to 3.00 Å. Since only two of these oxygen atoms have hydrogen atoms bonded to them¹⁴ only two hydrogen bonds can exist; thus the exact na-

Table II. Backbone Torsion Angles of Both Lasalocid A Ions of This Study and Those of the Lasalocid-Amine Complex¹³

	ion 1	ion 2	lasalocid-amine complex
O(1)-C(1)-C(2)-C(7)	-175.	170.	171.
C(1)-C(2)-C(7)-C(8)	0.	-7.	-7.
C(2)-C(7)-C(8)-C(9)	-86.	-75.	-77.
C(7)-C(8)-C(9)-C(10)	174.	176.	169.
C(8)-C(9)-C(10)-C(11)	-74.	-61.	-63.
C(9)-C(10)-C(11)-C(12)	172.	177.	-175.
C(10)-C(11)-C(12)-C(13)	170.	163.	-174.
C(11)-C(12)-C(13)-C(14)	-137.	-128.	-134.
C(12)-C(13)-C(14)-C(15)	82.	74.	84.
C(13)-C(14)-C(15)-O(6)	63.	73.	69.
C(14)-C(15)-O(6)-C(18)	155.	149.	142.
C(15)-O(6)-C(18)-C(19)	119.	109.	125.
O(6)-C(18)-C(19)-O(7)	62.	63.	75.
C(18)-C(19)-O(7)-C(23)	178.	176.	172.
C(19)-O(7)-C(23)-C(22)	61.	58.	65.
O(7)-C(23)-C(22)-O(8)	61.	62.	63.

ture of the hydrogen bonding scheme within this ion is unknown. In the other lasalocid A ion (ion I of Figure 4), a water molecule links O(2) to O(8) and the only intraionic hydrogen bond is between O(4) and O(8), 2.89 Å.

In both the previously reported monomeric and dimeric⁶ complexes with sodium ion, the cation coordination is remarkably similar. In each case five of the six coordinated oxygens are hydroxyl, O(4), carbonyl, O(5), ether oxygens, O(6) and O(7), and the hydroxyl, O(8), of a single lasalocid ion. The sixth coordination site varies in the three crystal structures and is provided by a methanol in the monomeric complex, by the carbonyl, O(5), of the other lasalocid ion in the head to head dimer, and by a carboxyl oxygen, O(1), of the other lasalocid ion in the head to tail dimer. In the solvated, sodium dimer of this study, one sodium ion has sixfold coordination while the other has sevenfold coordination. Sodium-oxygen distances are illustrated in Figure 4; the next shortest sodium-oxygen contact is a 2.93 Å distance between Na_{11} and O(5)₁₁. The sixfold coordination (Na_{11}) differs from any previously observed in that it is made up of four oxygen atoms of one lasalocid ion and both water molecules (Figure 4). The other sodium ion (Na_1) is coordinated by the same six oxygen atoms previously observed in the head to head dimer⁶ as well as one of the water molecules.

In addition to providing coordination for the sodium ion, the two water molecules are acceptors and donors of intracomplex hydrogen bonds, which are shown together with their distances in Figure 4. The water molecule (W_1) which is bonded to both sodium ions is involved in only one additional hydrogen bond

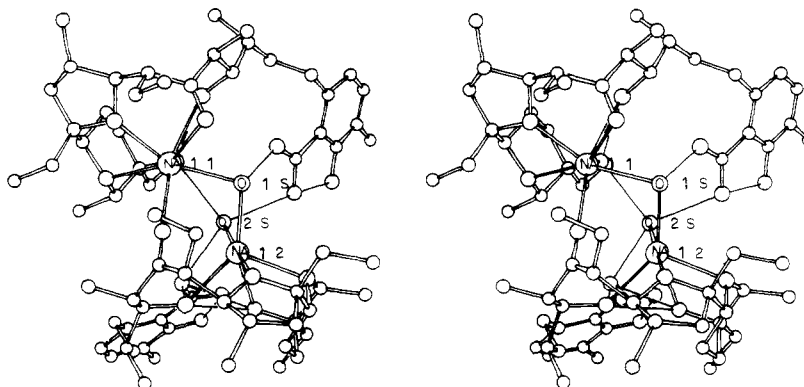


Figure 2. Stereodrawing of the dimer with hydrogen bonds illustrated as single lines: O1S and O2S are the two water molecules.

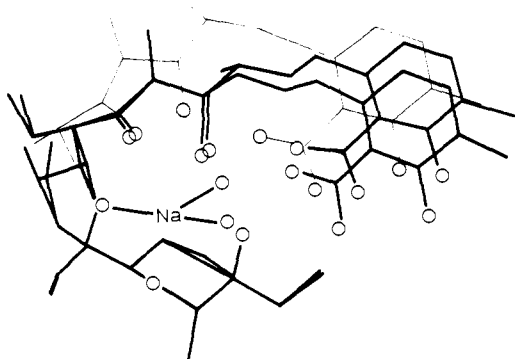


Figure 3. A comparison of the differences in overall conformation of the two independent lasalocid A ions (heavy lines) and of the lasalocid-amine complex¹³ (light lines) by a molecular least-squares fit.¹²

while the other water molecule (W_{II}), bound to only one of the sodium ions (Na_{II}), is involved in three hydrogen bonds, two of which are to ion I. Thus the effect of the two water molecules is to hold the two halves of the complex together through the intracomplex hydrogen bonding together with the coordination of the sodium ions. There are no hydrogen bonds which directly link one lasalocid A ion to the other and no hydrogen bonds exist between one dimer moiety and another.

Since each sodium ion can be considered to be associated primarily with a single lasalocid A ion, this dimeric complex can be thought of as the result of the initial association of the two monomeric sodium-lasalocid A units reported by Chiang and Paul.⁷ Through interactions with the water molecules, the two monomers are held together producing a conformationally stable intermediate in the complexation process. This complex appears to be a precursor to the head to head sodium-lasalocid A complex⁶ since the seven-coordinate sodium ion is already coordinated by the proper six oxygen atoms of the two lasalocid A ions. A facile transition pathway to the head to head dimer can be envisioned by the following concerted process: (1) a small change in the conformation of the backbone of ion II will bring $O(5)_{II}$ into a position so that it can bind to Na_{II} ; (2) through a rotation of one lasalocid A ion and its sodium ion with respect to the remainder of the complex, $O(5)_I$ can be coordinated to Na_{II} as both water molecules dissociate from the complex; and finally, (3) production of the two intraionic hydrogen bonds, $O(1)-O(8)$, to give rise to the observed structure of the head to head dimer.

It has been suggested that monomeric forms predominate at the surface of the membrane and are involved in uptake and release of ions⁷ while the dimeric forms are involved in the actual transport process. The structure observed in this study lends credence to this idea, provides hard structural data for the intermediate in the monomer-dimer conversion, appears to be a precursor to the head to head unsolvated dimer, and suggests a plausible mechanism for that conformational transition.

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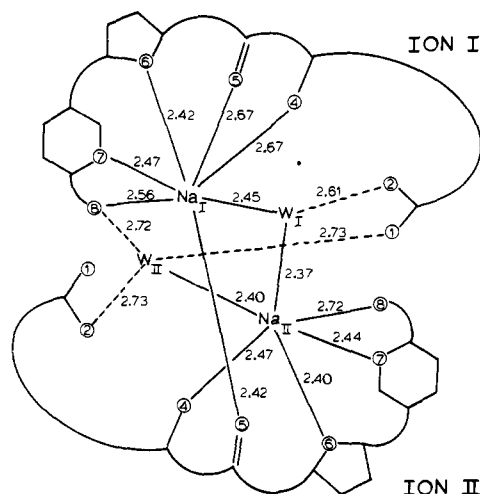


Figure 4. Schematic drawing of the complex illustrating the coordination of each sodium ion, the hydrogen bonding scheme to the water molecules, and the distances. Subscripts I and II distinguish one monomer unit from the other.

supervision of the data collection. This investigation was supported in part by Grant GM-19684 awarded by the National Institute of General Medical Sciences, DHEW. Figure 3 was drawn on PROPHEX, an NIH-sponsored biomedical computer network.

Supplementary Material Available: Listing of atomic coordinates, bond distances and angles, and a tabulation of observed and calculated structure factors (26 pages). Ordering information is given on any current masthead page.

References and Notes

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