Conformational Dynamics of the Carboxylic Ionophore Lasalocid A Underlying Cation Complexation–Decomplexation and Membrane Transport†

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ABSTRACT: The conformational dynamics of lasalocid A have been studied in a series of solvents of graded polarity by means of circular dichroism (CD) and computer-generated molecular models. In high polarity solvents, the uncomplexed anionic ionophore assumes an acyclic conformation minimizing intrinsic molecular strain energy. In this state, the dipoles of the liganding oxygens in the carbon backbone and the terminal carboxylate are stabilized by a high degree of solvent association. As the solvent polarity decreases, the dynamic conformational equilibrium progressively shifts toward a cyclic conformation which predominates at low polarity. Cyclization proceeds by rotation about three carbon–carbon hinge bonds. The resulting twist of the backbone introduces torsional strain which is offset at low polarity by electrostatic stabilization gained through intramolecular hydrogen bonding. Formation of a cation inclusion complex also stabilizes the cyclic conformer, even in relatively polar solvents. These observations suggest a scenario for carboxylic ionophore mediated transmembrane monovalent cation transport at the molecular level. The cation encounters an acyclic ionophore at the membrane interface where it ion pairs to the terminal carboxylate moiety, initiating formation of a lipophilic, cyclic cation inclusion complex. The complex, no longer constrained to the polar interface, diffuses across the membrane interior to the opposite face. There it reequilibrates with the polar environment, the ionophore reassuming the low energy, acyclic conformation and concomitantly releasing the enclosed cation. The free, acyclic ionophore is now confined to the opposite polar interface where it awaits the capture of a new cation to complete its catalytic transport cycle.

The extensive use of ionophores in experimental biology and technology directs attention to the cation complexation process that underlies ionophore-mediated membrane transport activity. The cation complexation reaction constitutes an exchange whereby the ionophore engulfs a cation, replacing the solvent molecules in the primary cation solvation shell with the liganding heteroatoms on the ionophore backbone (Diebler et al., 1969). This study provides a detailed analysis of the structural factors which control the conformational options and *monomeric* complexation reactions of a representative carboxylic ionophore, lasalocid A (X-537A, *lasalocid*).

The stability of a cation–ionophore inclusion complex is a function of the ability of the ionophore to prevent the cation from interacting with bulk solvent molecules. For the reaction

$$M^+ + I^- \rightleftharpoons [M^+I^-]^0 \quad (1)$$

where M^+ is a monovalent cation and I^- is an ionized carboxylic ionophore, complex stability can be expressed thermodynamically as the sum of several free energy terms:

$$\Delta G_{complex} = \Delta G_{M^+,desolv} + \Delta G_{I,desolv} + \Delta G_{I,conform} - \Delta G_{lig} \quad (2)$$

where $\Delta G_{M^+,desolv}$ is the energy required to transfer the cation from bulk solvent to the solvation sphere formed by the ionophore liganding cavity, $\Delta G_{I,desolv}$ is the energy necessary to desolvate the ionophore (Haynes & Pressman, 1974a,b), $\Delta G_{I,conform}$ is the energy required to convert the uncomplexed conformation of the ionophore to its complexed conformation, and ΔG_{lig} is the energy of interaction between the desolvated cation and the conformationally primed ionophore (Eisenman et al., 1968; Ovchinnikov et al., 1974; Pressman, 1976). The magnitudes of these terms for a given ionophore, in a given environment, determine its ability to bind various cation species and to discriminate between them.

The conformational options available to the carbon skeleton supporting the heteroatomic liganding groups determine the geometry of the ionophore inclusion complex which, in turn, determines the effectiveness of ligand focusing about the cation, i.e., the magnitude of ΔG_{lig}. In the case of the macrocyclic *neutral ionophores*, the covalently locked, cyclic conformation intrinsic to the molecule is predominant in determining ligand focusing (Bush et al., 1971). Cation complexation is accompanied by relatively small conformational changes and consequently relatively small changes in $\Delta G_{I,conform}$ (Fabbrizzi et al., 1978). However, the absence of covalent head-to-tail linkage in the *carboxylic ionophores* permits greater backbone flexibility and more profound conformational changes, and hence larger magnitudes of $\Delta G_{I,conform}$ accompany cation complexation.

Because of the greater flexibility of the carboxylic ionophores, their conformations respond strongly to environmental forces such as solvent polarity (Degani et al., 1973; Degani & Friedman, 1974; Painter & Pressman, 1979, 1980). The conformational effects produced by decreasing solvent polarity parallel the effects produced by the formation of ionophore–alkali cation inclusion complexes (Degani & Friedman, 1974; Anteunis, 1976; Patel & Shen, 1976; Shen & Patel, 1976). Thus, studying the effects of solvent polarity on solution conformation should provide insight into the structural factors that determine the ability of an ionophore to envelope a cation, as well as the effects of the membrane environment on the complexation process.

The presence of multiple asymmetric centers in the backbone of carboxylic ionophores confers chirality on their conformation. In the case of ionophores that contain discrete chromophores, e.g., lasalocid and salinomycin, chirality can be monitored by circular dichroism (CD) (Alpha & Brady, 1973; Degani & Friedman, 1974; Pressman & de Guzman, 1974; Occalowitz et al., 1976; Painter & Pressman, 1980). We have previously studied the effects of cation complexation and solvent polarity on the conformation of salinomycin utilizing

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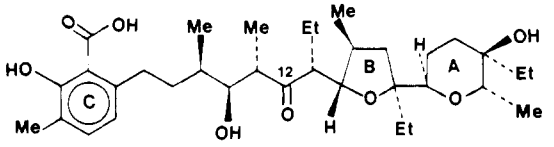

FIGURE 1: Structure of lasalocid.

CD signals arising from the medial C_{11} ketonic carbonyl (Painter & Pressman, 1979). These studies served as a prototype for the present study of lasalocid which contains two chromophores, namely, a terminal aromatic ring, ring C, and a medial C_{12} ketonic carbonyl (Figure 1). The presence of these strategically placed chromophores makes it possible to monitor conformational perturbations at two distant loci of the ionophore simultaneously. Computer analysis of the CD data, assisted by computer molecular modeling techniques, will be described.

Materials and Methods

Lasalocid and dihydrolasalocid (gifts from John Wesley, Hoffmann-La Roche) were purified prior to use. Lasalocid sodium salt was recrystallized from methanol (Berger et al., 1951). Dihydrolasalocid sodium salt was chromatographed on a Florisil column with a gradient of *n*-hexane/acetone (Westley et al., 1973). In both cases, the cation-free acidic forms of the ionophores were prepared by washing their cation complexes in diethyl ether with saturated aqueous citric acid. The organic layer was then washed 3 times with distilled water and flash evaporated. The crystalline product was vacuum dried.

CD spectra were obtained with a Cary Model 60 spectropolarimeter equipped with the Model 6001 CD accessory and a thermostated cell compartment. Absorption spectra were obtained with a Jasco UV-5 spectrometer. Scan speeds and time constants were adjusted to achieve suitable signal-to-noise ratios. The CD spectrometer was calibrated against aqueous *d*-10-camphorsulfonic acid (K & L Laboratories; twice recrystallized from acetic acid) assuming an $\epsilon_L - \epsilon_R$ of 2.20 ± 0.05 at 290 nm. CD data, recorded directly as degrees ellipticity, were expressed as molecular ellipticity, $[\theta]$ (Crabbé, 1967).

All solvents other than absolute ethanol were spectral grade. Tri-*n*-butyl-, tri-*n*-hexyl-, and tri-*n*-octylamines were vacuum distilled from powdered zinc prior to use. Tetramethylammonium and tetra-*n*-butylammonium hydroxides were heated under vacuum on a steam bath to remove all traces of volatile amines. The lasalocid and dihydrolasalocid anions were generated from the free acids by addition of 1.5 equiv of the desired amine or tetraalkylammonium hydroxide.

NaSCN and KSCN (Alfa Chemicals) were thoroughly dried prior to use in an Abderhalden apparatus. Titration of the anion with the appropriate alkali thiocyanate solution (standardized by flame photometry) yielded the molecular ellipticities and K_D values of the cation–ionophore complex. Saturation isotherms were plotted from linear computer fits of 1/[cation] vs. 1/$[\theta]$; the slope yielded K_D values while extrapolation of $[\theta]_I$ and $[\theta]_{II}$ to infinite cation concentration yielded $[\theta]_I$ and $[\theta]_{II}$ of the cation-saturated ionophore, respectively.

Resolution of peak II into its IIa and IIb component peaks was carried out on the Prophet Computer System (Raub, 1974) utilizing curve fitting and graphing procedures available within the system (Johnson, 1979; Marini & Perry, 1979).

All molecular modeling and manipulation of molecular structures were carried out on the Prophet Computer System by means of modeling programs within the Prophet program library (Rindone & Rush, 1980). Initially, a model of the lasalocid molecule was constructed from X-ray crystallographic coordinates (Johnson et al., 1970; Westley et al., 1970) of the "unprimed" lasalocid anion contained in the dimeric lasalocid A–Ba^{2+} complex. The conformation of the molecular model was refined by using a Fortran program, QCFF, external to the Prophet system (Warshel & Lifson, 1970; Warshel & Karplus, 1972; Huber & Warshel, 1974; Rohrer, 1980). This program utilizes consistent force-field calculations and assumes the equilibrium structure of the molecule to be that for which the energy of the electronic ground state, V_T, has a minimum value. V_T is calculated as the sum of intramolecular interactions arising from (1) bond stretching and compression, $V_{(r)}$, (2) bond angle bending, $V_{(\phi)}$, (3) bond torsion, $V_{(\theta)}$, and (4) nonbonded through-space interactions, $V_{(\rho)}$. The program provided the atomic coordinates of the energy minimized model as well as V_T, $V_{(r)}$, $V_{(\phi)}$, $V_{(\theta)}$, and $V_{(\rho)}$.

The purpose of the molecular modeling is to identify systematically all of the stereochemically reasonable solution conformations of lasalocid that may exist at the extremes of the polarity continuum in which the molecule was examined by CD. Several assumptions made in the calculations should be noted here. (1) The entire conformational space geometrically available to the molecule is not searched. Instead, it is limited by constraining backbone torsion angles to within ranges deduced from NMR coupling constants measured under appropriate solvent conditions (Schmidt et al., 1974; Shen & Patel, 1976, 1977; Patel & Shen, 1976; Anteunis, 1976). (2) In low polarity solvents, it is assumed that charge neutralization via intramolecular H bonding is predominant. This assumption primarily affects the stability of two intramolecular hydrogen bonds, $O_{26}^-\cdots HO_{40}$ and $O_{26}^-\cdots HO_{31}$. A third intramolecular hydrogen bond, the $O_{28}H\cdots O_{27}$ salicylate hydrogen bond, is extremely stable and polarity independent. The occurrence and stability of these hydrogen bonds in solution have been independently confirmed with NMR by monitoring the exchangeable hydroxyl protons as a function of solvent polarity (Patel & Shen, 1976; Shen & Patel, 1976). The $O_{40}H\cdots O_{26}^-$ and $O_{31}H\cdots O_{26}^-$ hydrogen-bonding distances in solution are assumed to be similar to those found in the unprimed half of the dimeric lasalocid–Ag^+ crystal complex, i.e., 2.71 and 2.94 Å, respectively (Mair & Paul, 1971). These bond lengths yield the cyclic conformer with the lowest V_T value. (3) Specific solvent effects are entirely ignored in apolar media. However, in high polarity solvents, changes in the rotamer populations of carbon chains containing heteroatomic substituents are known to occur due to specific solvent–heteroatom interactions (Suma, 1972). In order to accommodate lasalocid anion conformers in which there is enough space about backbone oxygen atoms and the terminal C_{25} carboxylate for the approach of polar solvent molecules, the van der Waals radius of each lasalocid oxygen atom was increased by 1 Å beyond its standard value (cf. Madison & Kopple, 1980; Pullman & Pullman, 1974). This assumption greatly increases the torsional strain, $V_{(\theta)}$, inherent to the polar, acyclic conformer and is included principally to make estimates of $\Delta G_{I,conform}(V_{(T),cyclic} - V_{(T),acyclic})$ more realistic and accurate.

The compatibility of CD signals arising from the C_{12} ketonic carbonyl (peak IIb) with the computer-generated lasalocid models was evaluated by means of the *octant rule* which provides a means of relating the optical activity of a carbonyl group to the asymmetry of its molecular environment (Moffitt et al., 1961; Crabbé, 1972). Operationally, this was accomplished by translating and rotating the Cartesian coordinate system used to identify the positions of the atoms in the model

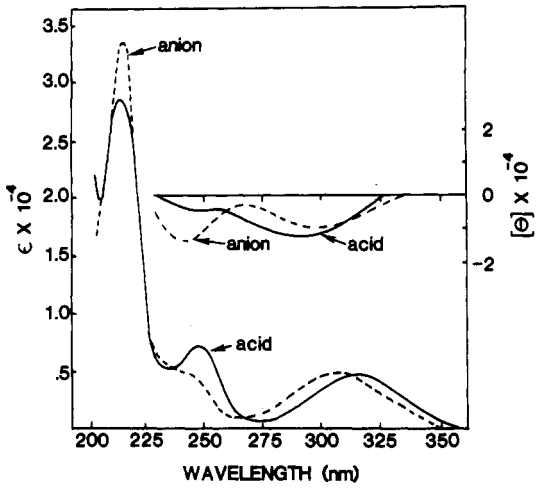

FIGURE 2: Absorption and CD spectra of protonated lasalocid (—) and lasalocid anion (---) in ethanol. The protonated form was stabilized by 0.5 equiv of HCl. The anion was generated by the addition of 1.5 equiv of tri-*n*-butylamine to protonated lasalocid.

from the molecular center of gravity to a position where the *xy*, *xz*, and *yz* planes correspond to the nodal and symmetry planes of the ketonic n→π* transition. The transformed Cartesian coordinates were then used to assign each ionophore atom to a specific octant. Subsequently, the cumulative effect of the atoms on the sign and magnitude of the CD peak was estimated.

Results

Figure 2 presents the UV absorption and CD spectra of lasalocid. The aromatic C ring gives rise to three electronic absorption bands characteristic of substituted benzene derivatives: (1) an $A_{1g} \rightarrow E_{1u}$ π→π* transition at 210 nm; (2) an $A_{1g} \rightarrow B_{1u}$ π→π* transition at 245 nm; (3) an $A_{1g} \rightarrow B_{2u}$ π→π* transition at 317 nm (nomenclature according to Duncan & Matsen, 1966). The 210-nm transition will not be treated in this study since it lies outside the transparency range of several of the solvent systems used.

The 245- and 317-nm π→π* transitions of the aromatic C ring of lasalocid generate CD bands at corresponding wavelengths. The position of the $A_{1g} \rightarrow B_{1u}$ π→π* absorption maximum at 245 nm corresponds to the CD maximum observed at 245 nm (peak I). The CD band corresponding to the 317-nm $A_{1g} \rightarrow B_{2u}$ π→π* absorption band (peak IIa) is contained along with the C_{12} ketonic n→π* transition (peak IIb) in the long wavelength CD composite peak centered at 294 nm (peak II), i.e., peak II in Figure 2 = peaks IIa plus IIb. Although the n→π* transition of the C_{12} ketonic carbonyl is too weak to appear in the UV absorption spectrum, it nevertheless generates a strong CD band. The presence of peak IIa within composite peak II can be easily confirmed by examining the C_{12} ketone reduction product of lasalocid (dihydrolasalocid; Westley et al., 1973), which lacks peak IIb (Figure 3).

In contrast to suggestions based upon X-ray crystal structures that it is the lasalocid dimer which transports cations (Chiang & Paul, 1977), investigations in phospholipid vesicles have clearly demonstrated free lasalocid anion to exist as a *monomer* within the polar head group region of the vesicle membrane prior to complex formation and that it is the 1:1 complex which transports monovalent ions across the membranes (Haynes et al., 1981). This is reinforced by the observation that only 1 in 10^4 transport events for lasalocid with Rb^+ is a current carrying event in the lipid bilayer, indicating charge carrying complexes of ionophore oligomers are not

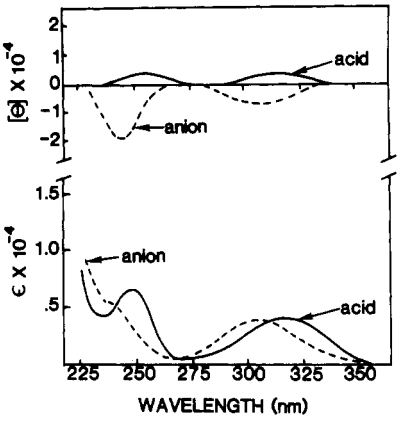

FIGURE 3: Absorption and CD spectra of protonated dihydrolasalocid (—) and dihydrolasalocid anion (---) in ethanol. The protonated form was stabilized by 0.5 equiv of HCl. The anion was generated by the addition of 1.5 equiv of tri-*n*-butylamine to protonated dihydrolasalocid.

prevalent (Mar & Pressman, 1972; Pressman, 1973). Therefore, the lasalocid species of prime consideration for ion complexation within membranes is the monomeric *free anion*.

Formation of the anion in vitro by deprotonation of the C_{25} carboxyl with base results in substantial changes in the absorption and CD spectra from that of the protonated form stabilized by 0.5 equiv of HCl (cf. Figure 2). The 245-nm absorption band observed for protonated lasalocid intensifies and shifts to 240 nm upon deprotonation while the 317-nm absorption band shifts to 310 nm with little change in intensity. The hypsochromic shifts observed for the absorption bands of lasalocid after ionization are analogous to those which occur following ionization of methylsalicylic acid and are attributed to differences between the ortho substituent effect exerted by the hydrogen-bonded pair $O_{26}^-\cdots HO_{28}$ vs. $O_{26}H\cdots O_{28}H$ (Degani & Friedman, 1974). In the CD spectrum, peak I shifts hypsochromically to 240 nm and intensifies upon deprotonation while peak II shifts bathochromically and diminishes in intensity.

The magnitudes of the CD transitions are highly solvent dependent. In order to correlate ionophore conformations with relevant solvent properties, it is necessary to quantify solvent–solute interaction at a molecular level. No single fundamental physical parameter uniquely predicts the ability of a solvent to interact with an ionophore. Properties beyond the classical *dielectric constant*, particularly the ability of the solvent to donate an electron pair or to hydrogen bond, must be considered. Kosower's *Z* values (Kosower, 1958) and Reichardt's E_T values (Reichardt, 1965) provide empirically effective parameters for correlating solvent polarity with ionophore solution conformation and ion complexing ability (Painter & Pressman, 1979–1981). We chose the extended scale provided by the E_T parameter for the current study. E_T values are available (Burgess, 1978) or can be calculated (Reichardt, 1965) for solvents extending through the entire polarity range encountered when proceeding across a biological membrane, i.e., from water (E_T = 58) to hexane (E_T = 30.5).

The molar ellipticities for peaks I and II, i.e., $[\theta]_I$ and $[\theta]_{II}$, of the lasalocid anion are shown as a function of solvent E_T value in Figure 4. $[\theta]$ decreases linearly for both peaks I and II between E_T values of 40 and 54. Above and below these values, $[\theta]$ of both peaks is relatively constant. Since ^1H and ^{13}C spin–lattice relaxation (T_1) studies indicate protonated lasalocid and its anion to exist as monomers in nonpolar solvents (cyclohexane, chloroform, and benzene) and in methanol (Patel & Shen, 1976; Shen & Patel, 1976; Lallemand & Michon, 1978), the E_T-dependent changes in $[\theta]_I$ and

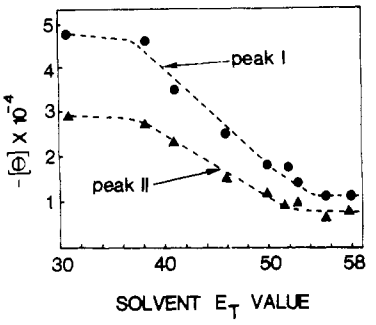

FIGURE 4: $[\theta]_I$ and $[\theta]_{II}$ of lasalocid anion as a function of solvent E_T value.

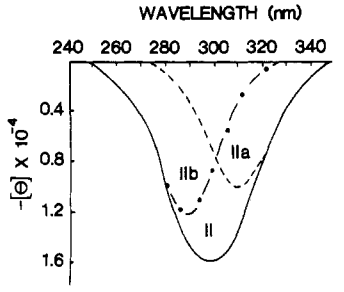

FIGURE 5: Deconvoluted peak II of lasalocid anion generated with tri-*n*-butylamine in acetonitrile. Peak IIa arises from aromatic ring C and peak IIb from the C_{12} ketone.

$[\theta]_{II}$ seen in Figure 4 must represent changes between the limiting conformational states of a monomeric lasalocid anion rather than conformational changes associated with a monomer–dimer equilibrium. An analogous polarity-dependent conformational change has been observed for salinomycin, a carboxylic ionophore that shows no propensity to dimerize (Painter & Pressman, 1979).

In order to determine the nature and loci of the solvent-mediated conformational change, it was necessary to resolve the components of peak II arising from the terminal aromatic C ring (peak IIa) and the medial C_{12} ketonic carbonyl (peak IIb). Peak IIb is a function of wavelength that can be satisfactorily approximated by a Gaussian curve of the form $A_i \exp[-k_i(\lambda - \lambda_i)^2]$, where A_i is the molar ellipticity, λ_i is the mean band wavelength, and k_i is a factor related to the standard deviation (Wellman et al., 1965). The IIa peak of anionic dihydrolasalocid A also appears Gaussian (see Figure 3) and is assumed to be similar in position and shape to that of lasalocid A anion under the same experimental conditions. It could, therefore, be used to predict limits for values of λ_{IIa} and k_{IIa} of a Gaussian model of the IIa peak of lasalocid anion. Peak II could then be resolved by considering it to be the superposition of two Gaussian bands such that

$$II = A_{IIa} \exp[-k_{IIa}(\lambda - \lambda_{IIa})^2] + A_{IIb} \exp[-k_{IIb}(\lambda - \lambda_{IIb})^2] \quad (3)$$

The values of A_{IIa}, A_{IIb}, k_{IIa}, k_{IIb}, λ_{IIa}, and λ_{IIb} were varied independently by computer until the position, extremum, and shape of the computer-generated curve II matched those observed experimentally (cf. Wellman et al., 1965; Miles & Urry, 1968). The relation of peak II to its component peaks IIa and IIb, for one representative set of conditions, is given in Figure 5.

$[\theta]$ values of the resolved IIa and IIb peaks are given in Table I. Changes in $[\theta]_{IIa}$ and $[\theta]_{IIb}$ independently parallel those observed for the composite peak II. $[\theta]_{IIa}$ and $[\theta]_{IIb}$ decrease linearly between E_T values of 40 and 54. Above and below these values $[\theta]$ is virtually constant for both peaks.

Table I: Resolution of Lasalocid Anion CD Peak II into Component Peaks IIa and IIb[a]

solvent	E_T	$-[\theta]_{II} \times 10^{-4}$	$-[\theta]_{IIa} \times 10^{-4}$	$-[\theta]_{IIb} \times 10^{-4}$
dioxane/water, 1:1	57.9	0.81	0.42	0.66
methanol	55.5	0.72	0.38	0.64
dioxane/water, 8:2	52.8	1.11	0.68	0.71
ethanol	51.9	1.08	0.65	0.85
dioxane/water, 9:1	49.7	1.24	0.94	0.96
acetonitrile	46.0	1.56	1.00	1.20
dichloromethane	41.1	2.34	1.28	2.00
chloroform	39.1	2.85	1.62	2.20
n-hexane	30.9	2.93	1.68	2.24

[a] Lasalocid anions were generated by the addition of 1.5 equiv of tri-*n*-butylamine.

Table II: Effects of Deprotonating Agents on the CD Spectrum of Lasalocid Anion in a Series of Solvents of Decreasing Polarity

base	MeOH, $-[\theta] \times 10^{-4}$	EtOH, $-[\theta] \times 10^{-4}$	CH_3CN, $-[\theta] \times 10^{-4}$	CH_2Cl_2, $-[\theta] \times 10^{-4}$
$(n\text{-Hex})_3N$				
I	1.20	1.98	3.10	3.84
II	0.76	1.16	1.70	2.44
IIa	0.44	0.75	1.12	1.40
IIb	0.64	1.00	1.20	2.00
$(n\text{-Oct})_3N$				
I	1.26	2.44	3.36	4.46
II	0.80	1.28	1.78	2.58
IIa	0.47	0.90	1.18	2.60
IIb	0.66	1.02	1.22	2.03
Me_4NOH				
I	1.16	1.40	1.90	*a*
II	0.66	1.04	1.40	*a*
IIa	0.43	0.50	0.68	*a*
IIb	0.64	1.00	1.18	*a*
$(n\text{-Bu})_4NOH$				
I	1.30	1.64	2.08	*a*
II	0.68	1.06	1.46	*a*
IIa	0.48	0.61	0.76	*a*
IIb	0.66	1.00	1.20	*a*

[a] These $[\theta]$ values could not be calculated in CH_2Cl_2 when tetraalkylammonium hydroxide bases were used to generate the C_{25} carboxylate due to chemical transformations arising from interaction of the base with the β-ketol system (C_{10}–C_{12}) of the ionophore.

In the course of comparing the effects of various deprotonating agents on the CD spectrum of lasalocid, we observed that different nitrogenous bases produced different effects on the magnitudes of peaks I and IIa while *peak IIb was base invariant* (see Table II). Base-induced changes in $[\theta]_{IIa}$ paralleled the base-induced changes in $[\theta]_I$ from which we conclude that the bases interact exclusively with the terminal chromophore, presumably by ion pairing with the C_{25} carboxylate. The lack of sensitivity of peak IIb to the base species confirms that ion pairing occurs without perturbing gross molecular conformation (Painter & Pressman, 1980).

Complexation of lasalocid anion with alkali cations on the other hand results in large, solvent-dependent increases in $[\theta]_I$ and $[\theta]_{II}$ (Figure 6 and Table III). The λ_{max} values for peaks I and II of the Na^+ and K^+ complexes are invariant at 240 and 290 nm, respectively. As the solvent E_T value drops and the dissociation constant decreases, the magnitudes of peaks I and II increase for both Na^+ and K^+ complexes.

The deconvoluted values of peaks IIa and IIb for the cation–ionophore complexes given in Table III were calculated

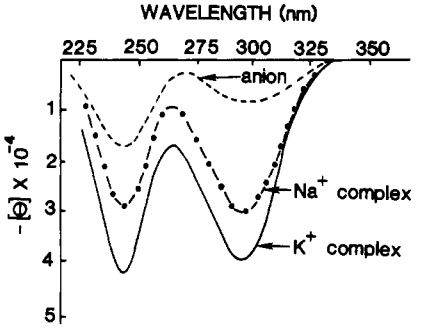

FIGURE 6: CD spectra of the Na^+ (···) and K^+ (—) complexes of lasalocid anion (---) in ethanol.

Table III: Effects of Alkali Cation Inclusion on the CD Spectrum of Lasalocid Anion in a Solvent Series of Decreasing Polarity

cation	CH_3OH, $-[\theta] \times 10^{-4}$	EtOH, $-[\theta] \times 10^{-4}$	CH_3CN, $-[\theta] \times 10^{-4}$	CH_2Cl_2, $-[\theta] \times 10^{-4}$
Na^+				
I	2.54	3.13	4.50	5.10
II	2.00	2.90	3.80	4.70
IIa	0.68	0.95	1.29	1.63
IIb	1.50	2.38	3.10	3.90
K^+				
I	3.40	4.80	5.20	5.36
II	3.20	4.40	4.80	5.00
IIa	1.18	2.00	2.60	2.50
IIb	2.74	3.80	4.20	4.50

in a manner analogous to that employed for peaks IIa and IIb of lasalocid anion. Cation complexation results in extremely large increases in $[\theta]_{IIb}$. As a consequence, peak II is dominated by the ketonic $n\rightarrow\pi^*$ transition, which accounts for λ_{max} of peak II remaining at 290 nm for both the Na^+ and K^+ inclusion complexes. Changes in $[\theta]_{IIa}$ upon cation inclusion parallel and are proportional to changes induced in $[\theta]_I$ upon cation inclusion and, although greater in absolute magnitude, resemble changes induced in the CD spectrum of lasalocid anion by decreasing solvent E_T value (compare Tables I and III). The larger changes observed in $[\theta]$ upon cation complexation probably arise to some extent from contributions to the rotational strength of the chromophores due to *dissymmetric placement* of the static charge of the complexed alkali ion within the ionophore complexation sphere (Urry, 1970). Nuclear magnetic resonance studies indicate that there are only minor adjustments in the solution conformation of free lasalocid accompanying sodium ion inclusion in apolar media (Patel & Shen, 1976; Anteunis, 1976). From these data, we infer that the conformer stabilized at low E_T values strongly resembles the conformer stabilized by alkali cation inclusion.

K_D values for the ionophore–alkali cation complexes were obtained from CD-monitored titrations with alkali thiocyanates (Table IV). With decreasing E_T values, K_D values are increasingly sensitive to the base species used to generate the lasalocid anion. The trialkylamines lead to K_D values 10–50 times greater than those observed for the tetraalkylammonium hydroxides in the same solvent. This reflects the poorer ability of the loosely ion paired tetraalkylammonium ion to block interaction between the ionophore and alkali ions compared to the more tightly held, hydrogen-bonded, trialkylammonium ion (Painter & Pressman, 1980). Thus, *data from the literature in which different amines and alkylammonium hydroxides were used to generate the ionophore anion are not strictly comparable* (Pressman, 1973; Pressman & de Guzman, 1975). It is equally inappropriate to employ LiOH as an anion-generating base (Degani & Friedman, 1974), since Li^+ has an affinity for lasalocid comparable to that of Na^+ (Pressman, 1969).

Discussion

Lasalocid anion has previously been examined by CD in a series of solvents of graded polarity (Degani & Friedman, 1974). However, the effects of polarity on the magnitudes of $[\theta]_I$ and $[\theta]_{II}$ presently reported (Figure 4) are significantly smaller than those reported by Degani and Friedman, particularly in low polarity solvents. We suspect the source of this discrepancy may lie in the different agents used to deprotonate lasalocid. Degani and Friedman used both LiOH and tri-*n*-butylamine. LiOH is specifically indicated as the base used to generate the lasalocid anion in methanol. However, the base used to generate the anion in the remainder of the solvents is not clearly indicated. Li^+ readily forms lipophilic inclusion complexes with lasalocid, comparable in stability to those formed with Na^+, which increase in stability as solvent polarity decreases (Pressman, 1969). Thus the alkali complex formed when LiOH is used to deprotonate lasalocid in various solvents can, in and of itself, cause a shift toward the cyclic conformer which is characterized by larger $[\theta]$ values (see below). In the present study, lasalocid anions were generated by using bulky trialkylamines or tetraalkylammonium hydroxides. The nitrogenous cations of the tetraalkylammonium hydroxides, or those formed by proton abstraction in the case of the trialkylamines, are too hindered to form true inclusion complexes with lasalocid. Although these cations ion pair to the terminal chromophore, no gross conformational changes are induced in the ionophore backbone.

The structural features of lasalocid anion which mediate its conformational responses to solvent polarity (E_T) were analyzed by computer modeling (see Materials and Methods). Under high polarity conditions, energy minimization yielded a set of energetically degenerate conformers whose backbone conformation differs significantly from the backbone conformation which predominates in apolar solution (see below) and in the crystalline state (cf. Duesler & Paul, 1982). These polar conformers, which differ only in the disposition of the aromatic C ring around the C_6–C_7 bond, represent the spatial

Table IV: K_D Values for K^+ and Na^+ Complexes of Lasalocid as a Function of Base

base	CH_3OH		EtOH		CH_3CN		CH_2Cl_2	
	K_D (Na^+) $\times 10^{-4}$	K_D (K^+) $\times 10^{-4}$	K_D (Na^+) $\times 10^{-4}$	K_D (K^+) $\times 10^{-4}$	K_D (Na^+) $\times 10^{-4}$	K_D (K^+) $\times 10^{-4}$	K_D (Na^+) $\times 10^{-4}$	K_D (K^+) $\times 10^{-4}$
$(n\text{-Bu})_3N$	32.1	2.46	0.835	0.112	0.072	0.072	0.284	0.068
$(n\text{-Hex})_3N$	28.6	2.40	0.778	0.099	0.500	0.073	0.304	0.061
$(n\text{-Oct})_3N$	30.0	2.94	0.800	0.100	0.477	0.075	0.274	0.069
Me_4NOH	13.2	1.00	0.037	0.0048	0.012	0.0018	*a*	*a*
$(n\text{-Bu})_4NOH$	14.6	1.24	0.041	0.0054	0.014	0.0024	*a*	*a*

[a] K_D values could not be calculated in CH_2Cl_2 when ammonium hydroxide bases were used to generate the C_{25} carboxylate due to chemical transformations of the β-ketol system (C_{10}–C_{12}) by the base.

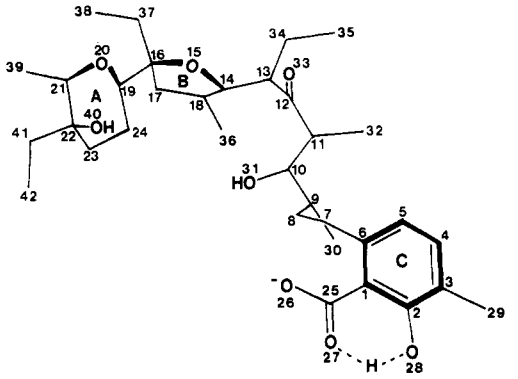

FIGURE 7: Acyclic quasi-linear conformer of lasalocid anion which predominates under high polarity conditions. This conformer has the lowest intrinsic molecular strain energy.

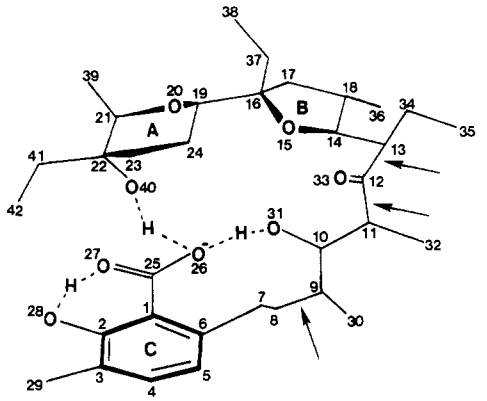

FIGURE 8: Cyclic conformer of lasalocid anion which predominates in apolar environments. Cyclization proceeds principally by rotation about the hinge bonds indicated by arrows. Dashed lines indicate hydrogen bonds.

arrangements of lasalocid having the lowest intrinsic molecular strain energy (Figure 7). As the aromatic ring is probably rotating rapidly about the C_6–C_7 bond at room temperature, the C_6–C_7 rotamers are spectroscopically indistinguishable.

Computer modeling indicates that the tetrahydropyranyl A ring of the acyclic conformer is in a chair conformation. The C_{22} ethyl group and the C_{19}–C_{16} bond joining ring A with ring B are equatorial, while the C_{22} hydroxyl group ($O_{40}H$) and the C_{21} methyl group are transdiaxial. The tetrahydrofuran B ring is in an envelope conformation with C_{18} lying below the plane of the other four atoms (C_{14}, O_{15}, C_{16}, and C_{17}). The aromatic C ring is planar. The C_{25} carboxylate, however, is twisted out of the plane of the ring very slightly (3°), minimizing unfavorable steric interactions with the ionophore backbone at C_6. The carbon–carbon backbone bonds lying between rings B and C, C_6 through C_{14}, are all in their lowest energy-staggered conformation, with torsion angles near 180°.

Under apolar modeling conditions (see Materials and Methods), the geometrical options available to the molecule are highly constrained, consistent with the large number of discrete NMR coupling constants which have been resolved in solvents with E_T values less than 40 (Schmidt et al., 1974; Shen & Patel, 1976; Anteunis, 1976; Shen & Patel, 1977). As a consequence, our energy minimization calculations quickly converged on a single cyclic conformer (Figure 8). Formation of the cyclic conformer proceeds without significant conformational changes in ring A, B, or C. The carboxylate is twisted out of the plane of the C ring 26° which is necessary to permit formation of the $O_{40}H\cdots O_{26}^-$ hydrogen bond adjacent to the steric bulk of the carbon backbone at the C_6 ortho position. The major conformational changes during cyclization occur by rotation around the C_8–C_9, C_{11}–C_{12}, and C_{12}–C_{13} carbon–carbon bonds. These three bonds play the role of the *hinge* bonds (Deber & Pfeiffer, 1976; Pfeiffer & Lardy, 1976) previously proposed for A23187. The C_7–C_8–C_9–C_{10} torsion angle rotates from anti to gauche which moves the C_{25} carboxylate into the vicinity of the $O_{31}H$ hydroxyl and O_{33} ketonic liganding moieties. The carboxylate is now in a position to form the $O_{26}^-\cdots HO_{31}$ intramolecular hydrogen bond. Cyclization is completed by reduction of the O_{33}–C_{12}–C_{11}–C_{10} torsion angle from 74° to 32° which focuses the AB ring system into the central liganding cavity and places the $O_{40}H$ hydroxyl in a position to form a second structure-stabilizing hydrogen bond, $O_{40}H$–O_{26}^-. Subsequently, van der Waals strain arising from 1,3 steric interactions between the C_{32} methyl group and the C_{34},C_{35} ethyl group is minimized by a decrease in the O_{33}–C_{12}–C_{13}–C_{14} torsion angle from 60° to 29°. It has been pointed out from comparison of various X-ray crystal structures that lasalocid adopts virtually the same conformation in all of its cation-inclusion complexes (Duesler & Paul, 1982). The torsion angles of the cyclic conformer are similar to the torsion angles found in the Na^+–lasalocid–water (2:2:2) crystal complex (Smith et al., 1978).

The rotation of the C_{25} carboxylate in the cyclic conformer 26° out-of-plane destroys the plane and center of symmetry of the aromatic chromophore, rendering it *dissymmetric*. Induction of dissymmetry establishes a diastereomeric relationship between the chromophore and the existing stereochemistry of the ionophore backbone. Since the backbone is capable of bending such that approach of the $O_{40}H$ to form the head-to-tail hydrogen bond with the C_{25} carboxylate is more favored from *above* the plane of the C ring [(+) rotation of the carboxylate] than from *below* [(−) rotation of the carboxylate], one of the two possible diastereomers predominates. As a result, dissymmetric contributions to the aromatic $\pi\rightarrow\pi^*$ transitions arising from the preponderance of the single diastereomer should be quite large, greatly outweighing those induced by asymmetrically disposed substituents. The magnitude of the $\pi\rightarrow\pi^*$ bands will consequently be dominated by the stereochemistry in the region of the carboxylate (Moscowitz et al., 1961; Djerassi et al., 1962; Urry, 1970). If the cyclic conformer depicted in Figure 8 exists in apolar solution, the $\pi\rightarrow\pi^*$ bands of lasalocid anion in low E_T solvents should be characterized by relatively large values of $[\theta]_I$ and $[\theta]_{IIa}$. Examination of Tables I and II shows that large values of $[\theta]_I$ and $[\theta]_{II}$ are indeed observed only under experimental conditions which are consistent with the modeling conditions used to generate the cyclic conformer. As solvent E_T values increase and the conformational equilibrium shifts toward the acyclic conformer (Figure 7) in which the aromatic chromophore is virtually symmetric, the magnitudes of $[\theta]_I$ and $[\theta]_{IIa}$ drop accordingly.

Interaction of the C_{25} carboxylate with nitrogenous cations to form ion pairs results in changes in peaks I and IIa which are similar to those observed during cyclization (see Table III). The extent of perturbation of the CD bands reflects the size of the nitrogenous cation. Space filling and computer-generated models indicate that the carboxylate must rotate out of the plane of the C ring in order to permit the bulky cation to ion pair adjacent to the ionophore backbone. The trialkylammonium cations cannot, however, be directly compared with the tetraalkylammonium cations with respect to size due to differences in ion pairing modes (Painter & Pressman, 1980). Since the nitrogenous cation is itself achiral, the CD band perturbation must arise from the chiral ionophore

backbone directing the approach of the nitrogenous cation to the C_{25} carboxylate to favor formation of one particular diastereomer.

Resolution of peak IIb from composite peak II permits the direct observation of the effects of solvent polarity and cation inclusion on the conformation of the ionophore backbone. Two of the three principal rearrangements indicated by the computer models to accompany cyclization occur adjacent to the C_{12} ketone, rendering it a sensitive CD probe for correlating observed backbone conformations with the proposed high and low polarity conformers. Peak IIb becomes increasingly negative as the solvent polarity drops (see Table I) or upon inclusion of an alkali ion (see Table III). Application of the *octant* rule (Moffitt et al., 1961; Crabbé, 1972) to the computer-generated models provides a semiquantitative prediction of the effect cyclization should have on $[\theta]_{IIb}$ (see Materials and Methods). Cyclization of the acyclic conformer moves the AB ring system from a position straddling the front lower left and the front upper left octants fully into the front upper left octant. As a consequence, the cyclic conformer should have very large *negative* values of $[\theta]_{IIb}$, a condition which obtains in low polarity solvents and in cation inclusion complexes.

The conformational changes per se associated with cyclization are energetically unfavorable; the computer model indicates that $\Delta G_{I,conform}$ is about 10 kcal/mol. The unfavorable cyclization energy is generated principally by torsional strain introduced by twisting about the hinge bonds and nonbonded, through-space interactions between the C_{32} methyl and C_{34},C_{35} ethyl group. As solvent polarity drops, however, the stabilization of the carboxylate gained by formation of the intramolecular hydrogen bonds and the growing propensity for hydrophobic over hydrophilic interactions overcome the conformational energy barrier for cyclization.

The scenario presented superimposes dynamic conformational factors on the presently accepted mechanism of lasalocid-mediated monovalent cation transport (Pressman, 1976; Haynes et al., 1980) which presumably apply to carboxylic ionophore mediated monovalent cation transport in general. The transport cycle begins with the lasalocid A anion in an extended *acyclic* conformation confined to the membrane interface where it is stabilized by the polar environment in the lipid polar head group region [the polarity of the lipid polar head group region has been demonstrated to be similar to that of methanol, i.e., $E_T \geq 55.5$ (Haynes & Pressman, 1974a)]. Under these conditions, the liganding heteroatoms are strung out and consequently cannot present a concerted inducible dipole system capable of strong interaction with solution cations. The strength of a cation–dipole interaction between a given backbone liganding oxygen ($O_{31}H$, O_{33}, O_{15}, O_{20}, and $O_{40}H$) and a solution cation is inversely proportional to the *cube* of their separation distance while the strength of a cation–anion interaction between the C_{25} carboxylate and a solution cation is inversely proportional to the *square* of their separation distance (Feynman et al., 1964). Thus, ion pairing rather than ion-induced dipole interaction appears to be a more likely mechanism for formation of initial encounter complexes between the acyclic ionophore at the membrane interface and solution cations. Ion pairing represents the limit of interaction between the acyclic ionophore conformer and hindered cations such as the alkylammonium ions (see Table II). If equivalent ion pairs are formed with smaller alkali cations, localization of the cation on the C_{25} carboxylate makes ion-induced dipole interactions more favorable with neighboring liganding oxygens, and the resulting forces reorient the ionophore backbone about the hinge bonds into the cyclic conformer. As molecular reorientation proceeds, the heteroatoms move closer to the optimal ligand–cation bond distance permitted by structural constraints and form a liganding field capable of stabilizing the cation relative to the bulk solvent. The cyclic conformer with the polar liganding groups focused into the cation binding cavity and the lipophilic alkyl groups shielding the exterior is the form most compatible with the apolar membrane interior. Since this conformation has a reduced capability to interact with the polar environment at the membrane surface, it readily leaves the interface and enters the lipophilic membrane interior where it is further stabilized. Upon diffusion to the opposite membrane face, the complex is again subjected to a polar environment. Since electrostatic stabilization no longer supersedes the unfavorable ΔG of cyclization, the complex releases the cation and remains as the acyclic anion at the interface available for the next phase of its transport cycle.

It has long been recognized that environment affects ionophore complexation by altering the energy required to desolvate cations, $\Delta G_{M,desolv}$ of eq 2 (cf. Eisenman et al., 1968). It is now apparent that ionophore-mediated transport is also controlled in part by *environmental modulation of the conformation of the ionophore.*

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