tection have appeared in the literature (*4*, *6–8*). While adequate resolution of the aflatoxins is readily achieved, the detection sensitivity has been in the low nanogram range. The more sensitive technique of fluorescence detection, while successful for aflatoxins in the solid state, has met with difficulty in HPLC (*9*), since aflatoxin B_1, the most important from a toxic and carcinogenic viewpoint, has a poor fluorescence quantum yield in solution (*10*, *11*). A novel technique developed by Thorpe and Stoloff (*12*) obviates this problem. By the addition of trifluoroacetic acid (TFA), aflatoxin B_1 is converted to aflatoxin B_{2a} (*13*), which is known to be highly fluorescent in hydrogen-bonded solvents (*14*). Aflatoxin G_1 is also transformed into G_{2a}, but B_2 and G_2 are unaltered (*15*). All four aflatoxins can then be separated on a reverse-phase HPLC column and quantitated fluorimetrically.

We have adopted this procedure but have substituted hydrochloric acid for TFA to make the derivatives of B_1 and G_1, since TFA causes additional unidentified peaks to appear in the chromatogram. Our method consists of drying the aflatoxin-containing extract or standard under a stream of nitrogen, adding a few drops of 1*N* HCl, allowing 15 minutes for reaction, evaporating under nitrogen on a steam bath, and finally, redissolving the sample in elution solvent. This procedure is reported (*16*) to have a high yield (> 90 percent) of aflatoxin B_{2a} and G_{2a}, which is consistent with our observation of a single peak per aflatoxin eluting from the column. Mixtures of water and ethanol (75:25 by volume) are used to elute the aflatoxins on a μBondapak C_{18} column (*17*). The resolution as well as the retention time of the aflatoxins can be varied by changing the proportions of water and ethanol. Both solvents were of high purity (*18*).

Figure 2 shows some typical aflatoxin chromatograms obtained by using the above procedure and the laser fluorescence detection scheme illustrated in Fig. 1. Figure 2a represents 30 pg each of the derivative aflatoxins G_{2a} and B_{2a} and the aflatoxins G_2 and B_2. The absence of secondary peaks and the lack of baseline fluctuations are noteworthy. Figure 2b is a chromatogram of an extract prepared (*19*) from 10 g of yellow corn known to contain 7 ppb of aflatoxin B_1. At present, the limit of detection in the corn sample with this method is approximately 2 ppb of aflatoxin B_1, the sensitivity being limited by interferences from other compounds in the corn extract. However, with better cleanup procedures it is expected that this detection limit can be further reduced.

In quantitating aflatoxin contamination it is important to establish the linear dynamic range of the detector. By injecting known quantities of appropriately diluted standards and their derivatives onto the column we have established a linear response of more than three orders of magnitude, as shown in Fig. 3. Scatter in these data, especially at higher signal levels, is largely a result of inaccurate graphical integration of the peaks. The signal-to-noise ratio at the lower limit of detection, 750 fg, was roughly 2 to 1 with a 3-second time constant on the lock-in amplifier.

Since the aflatoxins are considerably diluted as they elute from the column, it is of interest to determine what quantity of aflatoxin is in the detection volume itself. We assume that the peak has a Gaussian profile, $f(x) = (2\pi)^{-1/2}\ \sigma^{-1} \exp(-x^2/2\sigma^2)$, where 3.56 σ represents the full width of the peak at half-maximum intensity (FWHM). At the detection limit, the FWHM is 0.4 minute at a flow rate of 1.5 ml/min, giving a value of $\sigma = 0.17$ ml. The normalization of a Gaussian profile is such that the integral of $f(x)$ over all x is unity. Hence, the concentration in femtograms per milliliter at the detection limit is $C(x) = 750 f(x)$, and the aflatoxin concentration at the maximum of the peak is $C(0) = 1800$ fg/ml ($6 \times 10^{-12} M$). Thus the amount of aflatoxin in the 4-μl detection volume is only 7 fg or about 1×10^7 molecules at the signal maximum, and the amount of aflatoxin in the laser beam is even less. Although still higher detection sensitivities have been obtained in gas-phase laser-induced fluorescence (*20*), the present study illustrates that laser fluorimetry of condensed media in conjunction with chromatographic separation can detect and quantitate extremely low levels of fluorescent species.

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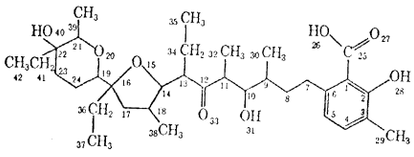
Monomeric Forms of the Acid Ionophore Lasalocid A (X-537A) from Polar Solvents

Abstract. *X-ray structural analyses have been carried out on the free acid of lasalocid A (X-537A) and on the sodium salt, both crystallized from methanol solution. In each case the structure is monomeric with one molecule of methanol complexing to the free acid and to the salt.*

Among acid ionophores, lasalocid A (X-537A) (**1**) has several unique features and has been the subject of many chemical and biochemical studies. Since the initial establishment (*1*) of the detailed structure and stereochemistry of lasalocid, x-ray studies have been carried out on the barium salt (*1*, *2*) and the silver salt (*3*) of lasalocid, the silver salt of 5-nitrolasalocid (*4*), two forms of the sodium salt of 5-bromolasalocid obtained from acetone and from carbon tetrachloride solutions (*5*), and the free acid of 5-bromolasalocid hemihydrate that had been crystallized from a hexane–methylene chloride solution (*6*). In these crystals, the conformation of the backbone of the ionophore has been essentially in-

variant (*7*) with most of the oxygen atoms directed to the interior and to one side of the roughly circular structure formed by "head-to-tail" hydrogen bonding of both O(40) and O(31) with the carboxyl (or carboxylate) groups. Lasalocid possesses the remarkable property, which sets it apart from almost all the other acid ionophores (*8*), that all of the structures studied in the crystal are dimers. These dimers have an almost entirely nonpolar exterior, with the metal ions (and, in the case of the free acid, a water molecule) being completely encapsulated by the two ionophore entities. Nuclear magnetic resonance (NMR) studies (*5*) in $CDCl_3$ and acetone-d_6 indicated that the backbone structure of the lasalocid entity as determined from the x-ray work was consistent with the NMR data, in that this structure did not appear to vary with solvent and that, in nonpolar solvents, dimeric structures similar to that found in the crystalline form I of the Na^+ salt of 5-bromolasalocid were present. A more comprehensive NMR study of lasalocid in nonpolar solvents (*9*) confirmed these findings,



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while an examination in polar solvents (*10*) indicated that monomeric forms largely predominate in such an environment. We now report the structures of crystals of the sodium salt of lasalocid A and of lasalocid A itself, both grown from methanol solution, and we demonstrate that under these conditions the structures are indeed monomeric.

Crystal data for un-ionized lasalocid A obtained from methanol (*11*, *12*) are: $C_{34}H_{54}O_8 \cdot CH_3OH$; molecular weight ($M$) = 622.6; system, orthorhombic; unit cell parameters, a = 26.628(10), b = 13.014(5), and c = 10.609(3) Å; unit cell volume (V) = 3676 Å^3; molecules per unit cell (Z) = 4; calculated density (ρ_{calc}) = 1.13 g cm^{-3}; total electrons per unit cell [F(000)] = 1360; and space group, $P2_12_12_1$. The data for the sodium salt obtained from methanol are $C_{34}H_{53}O_8^- \cdot Na^+ \cdot CH_3OH$; M = 644.9; orthorhombic; a = 20.202(2), b = 17.678(2), and c = 10.221(1) Å; V = 3650 Å^3; Z = 4; ρ_{calc} = 1.17 g cm^{-3}; F(000) = 1400; and space group, $P2_12_12_1$. The structures of both compounds were determined by direct methods (*13*) and were refined to conventional agreement index (R) values of 0.048 on 2683 nonzero reflections and of 0.044 on 2943 nonzero reflections for the free acid and sodium salt, respectively. In both structures, all the hydrogen atoms of the lasalocid entity, including hydroxyl hydrogens, could be clearly located, as could the hydroxyl hydrogen atoms of the methanol molecules (*14*). The location of all the hydrogen atoms provided the first opportunity to establish unequivocally the hydrogen bonding scheme in lasalocid or a derivative (*15*).

Stereoscopic views of the structures of the free acid and of the sodium salt are shown in Fig. 1. The structures of the lasalocid portions in the two crystals are quite similar, each possessing the familiar head-to-tail hydrogen bonding. In the case of the free acid, O(26) acts as a hydrogen bond donor to O(40), while both O(40) and O(31) act as hydrogen bond donors to the methanol molecule; the methanol hydroxyl hydrogen forms a hydrogen bond with O(15). In this form of the free acid, the ketone, O(33), is not involved in hydrogen bonding. This finding is consistent with the recent spectroscopic data of Rousseau and colleagues (*12*). In the sodium salt, the sodium ion is complexed by the same five oxygen atoms [O(15), O(20), O(31), O(33), and O(40)] that complexed the Na^+ ions in the dimeric forms (*5*); there is no Na^+---O(carboxylate) coordination. The other "side" of the Na^+ ion is coordinated to the oxygen of the methanol molecule, which partially plays the role of capping the cation as described previously for the dimeric sodium salts (*5*). The hydrogen bonding scheme has both O(31) and O(40) acting as donors to the carboxylate anion [O(26) and O(27), respectively]; in addition, the methanol forms a hydrogen bond to O(26). This finding, along with other spectroscopic data (*16*), demonstrates that a recent conclusion by Phillies and Stanley (*17*), namely, that aliphatic hydroxyl groups are not involved in hydrogen bonding in the sodium salt of lasalocid A, is incorrect.

While the gross structure of the lasalocid molecule does not change on complex formation with the Na^+ ion, there are small changes (11° to 22°) in the torsion angles about the C(6)-C(7), C(7)-

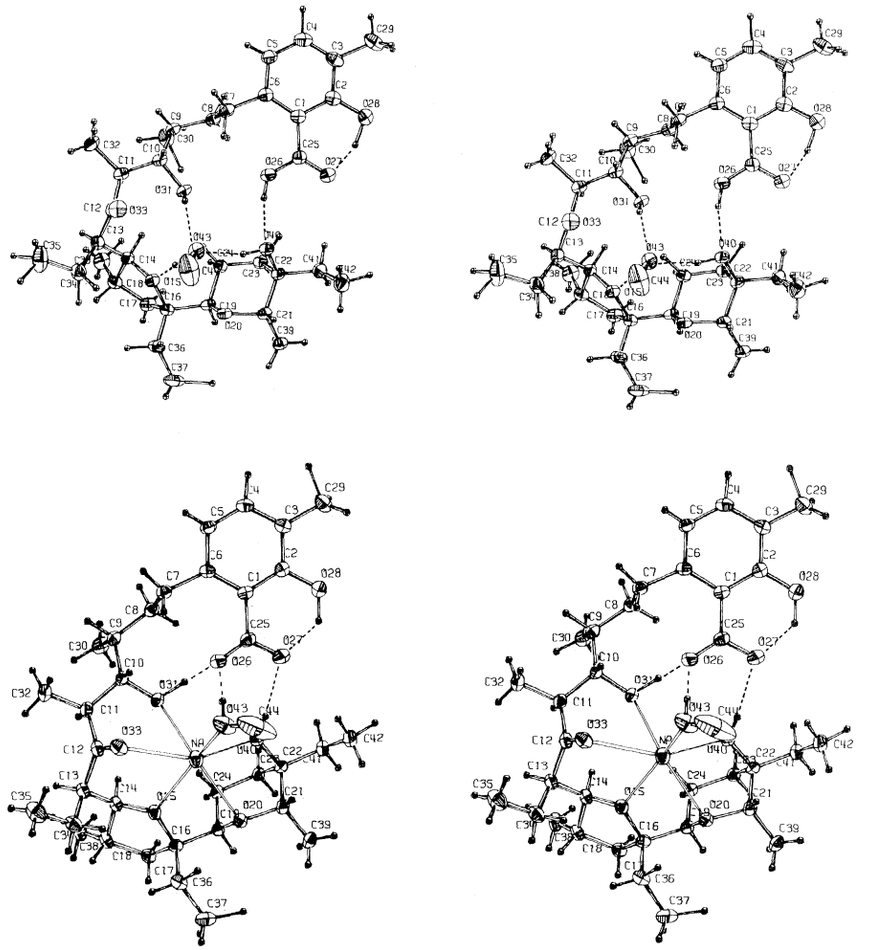

Fig. 1. (Top) Stereoscopic view of the 1:1 lasalocid : methanol complex. (Bottom) Stereoscopic view of the sodium salt of lasalocid with complexed methanol. Hydrogen bonds are shown by dashed lines, while Na^+---O coordination bonds are shown by light continuous lines.

C(8), C(8)-C(9), C(11)-C(12), C(12)-C(13), and C(13)-C(14) bonds that result in bringing O(33) closer to O(15), O(20), and O(40) and [with a major change in the position of O(43)] produce a very distorted octahedron of oxygen atoms around the sodium ion (*18*). Therefore, our results show that, unlike the case of neutral depsipeptide valinomycin (*19–21*), which complexes metals with a greatly changed conformation, the lasalocid molecule undergoes only minor changes in conformation on complex formation to sodium ions. This finding is consistent with results obtained previously for both monensin (*22, 23*) and grisorixin (*24, 25*).

The study reported here provides the following important information on lasalocid A. (i) There is no reliable evidence for a conformation of the lasalocid backbone significantly different from the circular one stabilized by hydrogen bonding that has been found in all x-ray studies thus far reported. Crystals obtained from the polar methanol solutions have now been shown also to contain this conformation. (ii) This appears to be the first time that the hydrogen atoms can be clearly recognized from an x-ray study of a lasalocid derivative, and so the hydrogen bonding patterns that were previously inferred are now confirmed. (iii) In contrast to our previous work, the x-ray analysis reveals a lasalocid A monomer (*26*). That lasalocid and its sodium salt crystallize from methanol solution as monomers is entirely consistent with the NMR results in polar solutions (*10*), and implies that the form in which lasalocid crystallizes is highly dependent on the species existing in the solution. The existence of monomeric and dimeric structures for lasalocid derivatives under different conditions implies that metal uptake and release in polar environments involve a monomeric form, while transport in nonpolar media takes place by means of a lasalocid dimer (*27*). This idea can be extended to suggest that the ionophore is a dimer in the lipid bilayer, but reverts to a monomeric structure at the polar exterior.

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8. A dimeric structure has been found in the case of the Ca^{2+} salt of the acid ionophore, A 23187 [G. D. Smith and W. L. Duax, *J. Am. Chem. Soc.* **98**, 1578 (1976)]. However, no examples, other than of lasalocid, are known of dimers with monovalent cations, or of uncharged species.
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14. In both crystals, there is slight disorder of the methanol molecule occasioned by libration of the methyl group while the position and direction of the hydrogen-bonded O-H group are preserved. This libration has prevented us from locating the methyl hydrogen atoms with certainty.
15. We have also completed the x-ray analysis of the 1 : 1 complex of 5-bromolasalocid with ethanol. This structure is almost identical, including hydrogen bonding assignments, with that of the 1 : 1 lasalocid : methanol complex described here, and hence it will not be described at this time (C. C. Chiang and I. C. Paul, unpublished data).
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26. It is of considerable interest that the free acid of a bromo derivative of isolasalocid, where the tetrahydropyran ring in lasalocid is replaced by a second tetrahydrofuran ring, has been shown to be monomeric and to exhibit a very different conformation [J. W. Westley, J. F. Blount, R. H. Evans, Jr., A. Stempel, J. Berger, *J. Antibiot.* **27**, 597 (1974)].
27. A numbering scheme for lasalocid and other ionophores based on biogenetic considerations has recently been proposed [J. W. Westley, *J. Antibiot.* **29**, 584 (1976)]. However, as all the published x-ray data refer to a scheme proposed originally by us, we continue to use that scheme for consistency and ease of comparison among the various compounds studied.
28. This work was supported by NIH grant GM 19336. The SYNTEX x-ray diffractometer and EXTL computer system used in this work were purchased with the aid of NSF equipment grant MPS 75-05911 to the University of Illinois. J. Berger and J. W. Westley of Hoffmann-La-Roche have generously provided us with many samples of lasalocid over the past few years. We have benefited greatly from discussions with Dr. Westley and with D. J. Patel and D. Rousseau of Bell Laboratories. Dr. Rousseau provided us with the crystalline samples used in this study.
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Anoxic, Hypersaline Basin in the Northern Gulf of Mexico

Abstract. *A 400-square-kilometer depression in the continental slope of the northern Gulf of Mexico (approximately 27°N, 91°W) has been found to contain anoxic, hypersaline (~ 250 grams per kilogram) water in the bottom 200 meters. The interface between the brine and overlying seawater acts as a midwater seismic reflector similar to those seen in the Red Sea. The bulk chemical composition of the brine is similar to that from the Red Sea, but differences between the two in both heat content and geomorphological setting indicate different modes of origin.*

In November 1975, sediment samples were collected from several of the larger bathymetric depressions in the continental slope of the northern Gulf of Mexico off Texas and western Louisiana from the R.V. *Gyre*. Sediment from the largest and most eastern of these basins was found to have an interstitial fluid containing nearly 150 g of chloride ion per kilogram, almost eight times that of seawater. Although similar hypersaline conditions have been found in samples collected from deep boreholes in the Gulf of Mexico (*1*) and in bottom waters from three Red Sea basins (*2, 3*), to our knowledge they have not been previously reported in any open continental shelf or slope environment.

Recently, the R.V. *Gyre* returned to this basin, centered at 26°55′N, 91°20′W (see inset, Fig. 1), in order to define the extent and nature of the brine conditions. A depression containing a pool of anoxic and hypersaline water which approaches the Red Sea brine in total salinity and which is at least three times greater in volume (*2, 3*) was mapped and sampled in detail. This initial report on a unique marine feature—tentatively named Orca Basin—presents the bathymetric, hydrographic, and seismic description of this basin as well as the bulk chemical composition of its brine.

Orca Basin is an intraslope depression situated within the complex described by Lehner (*4*) as "the growing margin of the Gulf Coast geosyncline." The complex bathymetry of the slope area has been attributed to salt diapirism and resulting slump features. The recovery of salt from boreholes drilled on several prominent highs along the slope (as close as 60