Lasalocid crystallized from Methanol: Spectroscopic and X-Ray Structural Evidence for Two Structures

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Results of i.r. absorption and Raman scattering data and X-ray crystallographic data for lasalocid crystallized from methanol have enabled identification of two distinct structures for the 1 : 1 lasalocid–methanolate complex. Both have space group $P2_12_12_1$ but possess a different molecular geometry. The ketone carbonyl stretching frequency in the 1 700—1 730 cm^{-1} region is a sensitive indicator of crystalline modification.

THE ionophore, lasalocid (X-537A) has been extensively studied recently owing to its antibacterial effect against coccocidial infection in chickens [1] and its therapeutic potential for cardiovascular disorders.[2] The ability of lasalocid to complex cations and mediate their transport across membranes is central to its biological activities. In an attempt to gain a better understanding of the biological function of lasalocid, we initiated a vibrational spectroscopic study of the ionophore using the techniques of Raman scattering and i.r. absorption. A Raman scattering investigation of lasalocid and its sodium complex was made by Phillies and Stanley [3] who found that similar conformations were present in solution and in solids. However, conclusions that these authors drew regarding the lack of hydrogen-bonding in the sodium complex were shown to be incorrect from spectroscopic [4] and X-ray [5] studies.

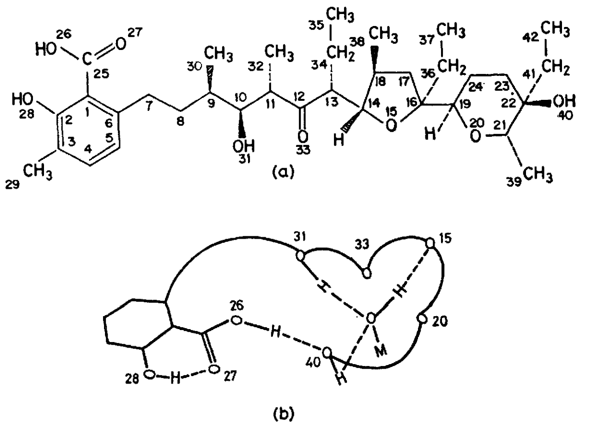

FIGURE 1 a, Chemical structure of lasalocid showing crystallographic atom numbering system, and b, conformation of lasalocid–methanolate complex

The molecular structure of lasalocid consists of a salicylic acid group attached to a hydrocarbon backbone terminating in a tetrahydropyran ring as shown in Figure 1.[6] It has been shown that in both solution (by n.m.r. studies),[7] and the solid (by X-ray studies) [5,8] the free acid of lasalocid assumes a folded cyclic structure stabilized by hydrogen bonding involving the O(31) and O(40) hydroxy-groups and the carboxylic acid group. At one side of this cyclic structure is a hydrophilic pocket, lined with oxygen; the other side is hydrophobic. Dimeric structures for metal complexes of lasalocid have been found in non-polar solutions [7] and in the crystals,[9] and a dimeric structure has been found for the free acid of 5-bromolasalocid monohydrate in crystals obtained from non-polar solvents.[8]

We report here the results of a spectroscopic and an X-ray crystallographic study on the free lasalocid. We found that the vibrational spectrum is very sensitive to structure, and that two different crystalline modifications of the 1 : 1 methanolate of lasalocid can be obtained from methanol solution. The spectroscopic differences in these two forms are discussed in relation to the determined crystal structures.

EXPERIMENTAL

To obtain single crystals for the Raman and the X-ray studies, powdered or polycrystalline material was dissolved in solvent and allowed to crystallize by cooling the solution. Solutions, prepared by directly dissolving the material in solvents, and glasses, prepared by quickly evaporating the solution on a silver chloride disc, were used in the i.r. studies. Raman spectra were obtained by use of a SPEX 1401 Double Monochromator, and Coherent Radiation 52 and 53 argon ion lasers. I.r. spectra were recorded on a Beckman IR 12 spectrophotometer.

RESULTS AND DISCUSSION

The i.r. absorption and the Raman scattering spectra of the free acid lasalocid are very complicated. Although we have examined the entire spectrum and find many features which change with conditions, we consider the ketone stretching frequency to be the most useful indicator of structural information owing to its isolation, strength, and clear frequency shifts.

By monitoring the frequency of the ketone stretching frequency in Raman spectra we have found evidence for two crystalline modifications (labelled forms A and B) of the 1 : 1 lasalocid methanolate complex (Figures 2a and b). The two spectra show several clear differences. The most pronounced is the position of the ketone carbonyl stretching frequency mentioned above which we assigned as the 1 724 cm^{-1} band in the crystal of form A and the 1 713 cm^{-1} band in the crystal of form B. We were unable to determine conditions which favoured one modification over the other.

I.r. data obtained from mulls of single crystals of A or

B showed corresponding differences in the ketone carbonyl stretching frequency (Figures 3a and b). As in the Raman spectrum, form *A* exhibits a higher (1 719 cm^{-1}) and form *B* exhibits a lower frequency (1 710 cm^{-1}). These i.r. frequencies differ slightly from those observed in the Raman spectra. These differences probably result from the different sample regions probed by the two techniques. In the Raman experiments the scattering takes place primarily from the bulk of the crystal. In contrast, large pieces of crystal in the i.r. sample attenuate all the light and only optically thin pieces of crystal are capable of resulting in a resolvable absorption spectrum. Our i.r. samples contained a mixture of optically thick and thin crystals because we did not want to grind the mulls extensively and break up the structure.

I.r. spectra of lasalocid glasses, made by rapid evaporation from methanol solutions, and of lasalocid in methanol solution were also obtained (Figures 3c and d). These spectra demonstrate that the shape and position of the ketone stretching band is dependent on the phase of the material. The ketone carbonyl stretch has a bandwidth of *ca.* 10 cm^{-1} in crystals of form *A* and *ca.* 20 cm^{-1} in the glass and solution spectra. A carbonyl stretch at the low frequency was found for both the solution and the glass samples, independent of the crystal form of the starting material.

I.r. and Raman spectra were also obtained for samples in which the labile protons were deuteriated. No detectable shifts were observed in the ketone carbonyl stretching frequency region confirming crystallographic results which show no hydrogen bonding involving the ketone group. There were many changes in other parts of the spectrum which proved to be very complex and difficult to interpret. The results of these experiments will be published elsewhere.[10]

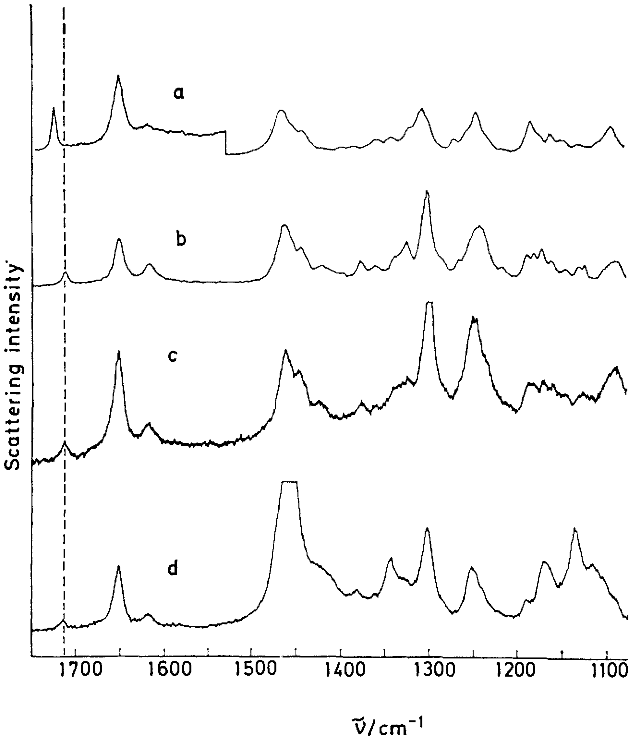

FIGURE 2 Raman spectra of lasalocid crystals grown from: a and b, methanol (forms *A* and *B* respectively); c, ethanol; and, d, propan-2-ol

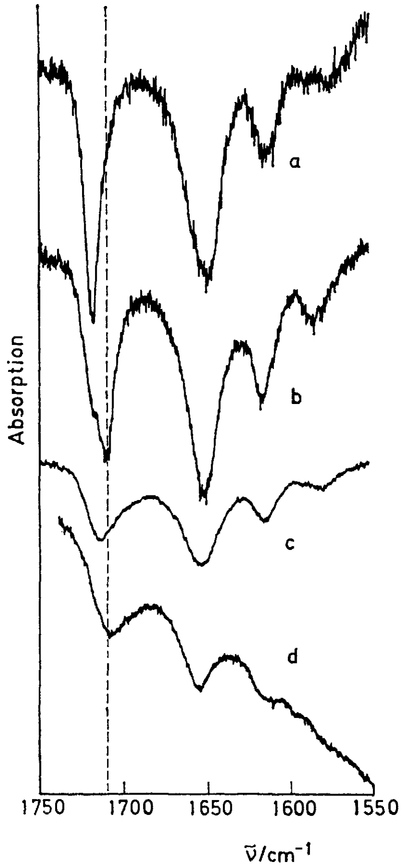

FIGURE 3 I.r. spectra of the C–O stretching region of lasalocid samples: a and b, mull spectra of forms *A* and *B*; c, spectrum of glassy sample obtained from methanol; and d, spectrum of solution of lasalocid in methanol

Figure 2c and d shows Raman spectra of crystals grown from ethanol and propan-2-ol. The morphology of the ethanol crystals was similar to that of crystals of form *B*; the crystals grown from propan-2-ol were very small and powdery. Each spectrum is very similar to that of crystal *B* although propan-2-ol bands near 1 460 cm^{-1} are unexpectedly strong in that crystal. By comparison of the positions of the bands in the spectra, however, we believe each of these crystals has the same molecular arrangement as that in crystals of form *B*. (There appears to be a discrepancy between the spectroscopic and *X*-ray work here. While spectra indicate that the lasalocid ethanolate crystals are similar to form *B*, *X*-ray structure analysis on the 5-bromolasalocid–ethanolate shows it to be isostructural with form *A*. However, it is possible that there are multiple types of crystals for each of the ethanolates, and it should not necessarily be assumed that the behaviour of lasalocid would be identical with that of 5-bromolasalocid.)

We have carried out a full *X*-ray structural analysis of both crystalline forms *A* and *B*, and the stereoscopic views of the molecular structure are shown in Figure 4.* Both crystalline forms are orthorhombic and belong to the space group $P2_12_12_1$. The principal difference in molecular geometry is in the change in orientation of the C(41)–C(42) ethyl group by *ca.* 120° about the C(22)–C(41) bond. Otherwise, the molecular structures are similar as are the intramolecular hydrogen-bonding arrangements. In both forms the methanol molecule is held in the same oxygen-lined pocket with the same hydrogen bonds [O(43)–H · · · O(15), O(31)–H · · · O(43), and O(40)–H · · · O(43)]. Between one form and the other there is a very slight tilt of the lower half of the molecule (that with the five- and six-membered oxygen-containing rings) with respect to the upper half (the aromatic ring). However, the O · · · O distances within the lasalocid portion of the complex remain the same, within 0.15 Å; the methanol oxygen atom retains its position with regard to the lower half, but there is a much greater difference (0.42 Å) in the distance of the methanol [O(43)] from the carboxyl oxygen [O(26)].

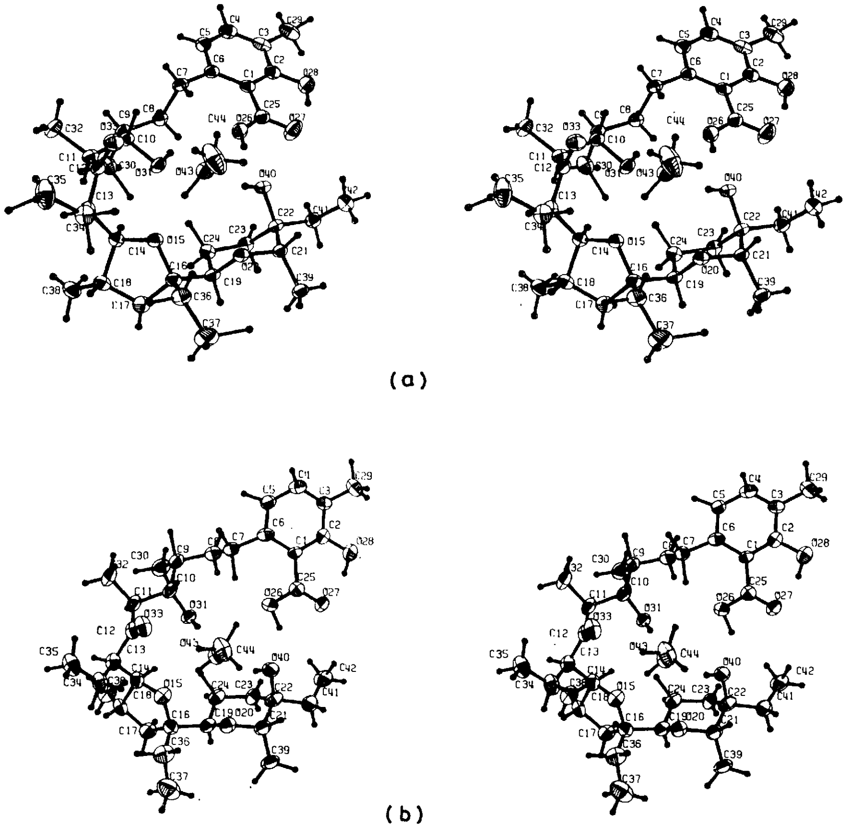

FIGURE 4 Stereoscopic views of the two lasalocid–methanolate structures: a, form *A* and b, form *B*

On the basis of the data presented we may draw several conclusions regarding both the structure of lasalocid and the application of Raman scattering to such systems. The occurrences of the two forms from methanol are about equally probable and we have grown several crystals of each, but are unable to determine the conditions which favour one structure over another. However, during 1—2 years crystals of form *B* in its mother liquor changed into form *A*. The vibrational spectrum serves as a useful probe of the structure and conformation; and the ketone carbonyl stretching vibrational mode is sensitive to both physical state and crystalline modification. Our data demonstrate that the spectroscopic differences we observe are independent of the presence or absence of bound solvent molecules and instead reflect small conformational changes of the lasalocid molecule.

While it must be recognized that there may be other more significant differences in the spectra of forms *A* and *B* than that in the ketone carbonyl stretch, only this mode can be readily assigned. It is worthwhile to attempt to find an explanation for the behaviour of the mode from the structural results. There is certainly no dramatic change in the intramolecular environment of the O(33) atom; in form *A* there may be a slightly closer approach of HO(31), but the H · · · O distance is still 2.53(5) Å and the O–H bond is roughly parallel to the C=O bond so it is doubtful that even a slight bifurcation of the hydrogen-bonding interaction could be invoked. In form *A* the ketone oxygen is involved in a contact (3.4 Å) with a C(30) from a neighbouring molecule; while

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* Cell dimensions, atom co-ordinates, thermal parameters, and observed and calculated structure factors are available as Supplementary Publication No. SUP 22429 (49 pp., 1 microfiche). For details see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1978, Index issue.

this contact is equal to the sum of the appropriate van der Waals radii, *it* is 0.2 Å shorter than any corresponding contact in form *B*. The C(30) atom is positioned such that any steric compression from this contact would tend to shorten the C=O bond and thus raise the stretching frequency. However, the frequency variation could reflect an electronic interaction between O(33) and a neighbouring group giving rise to a change in the C=O bond electron density.

Our vibrational spectroscopic studies of lasalocid very clearly indicate the importance and utility of such studies as well as some of the limitations. When molecular structural differences occur, even geometrically very minor ones, they may be readily detected with these techniques and therefore i.r. and Raman spectroscopic studies may serve, as here, as a useful preliminary step to a crystallographic study. In addition, certain recognizable bands may be particularly sensitive to structural changes and may function as clear structure 'indicators.' On the other hand, it is exceedingly difficult to assign all the modes in a complicated spectrum and in a case such as this where there are many hydrogen bonds and possibilities for resonant interactions, deuteriation may not clarify the assignments.

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