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Complexes of Amine Cations with Lasalocid A, a Microbial Ionophore

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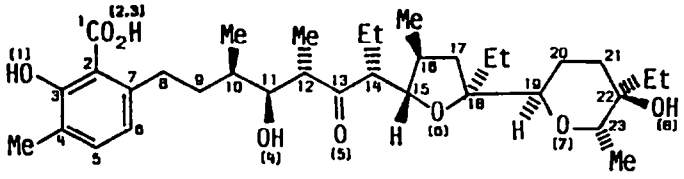
Abstract - Complexes having the stoichiometry [R-NH_3^+][lasalocid A^-] have been prepared and characterized, where R represents a series of organic substituents with diverse steric and electronic properties. The crystalline complexes dissolve readily in solvents of low polarity such as chloroform and are characterized in this solvent by molecular weight data and ^{13}C nmr spectroscopy. NMR data indicate that the cations bind the lasalocid A anion *via* hydrogen bonds to O_3, O_6, and O_8. Molecular weight data show that none of these complexes are appreciably dissociated in chloroform solution.

Lasalocid A (I)[1], a carboxylic ionophore produced by microorganisms, is capable of transporting mono-, di-, and trivalent metal cations across natural and artificial membranes.[2-4]



I

Crystallographic studies[5-9] of the Na^+, Ag^+, and Ba^{2+} complexes of the lasalocid A anion, hereafter abbreviated LAS, reveal that in all but one case, the cations are sandwiched by two LAS ligands, each of which has a cyclic conformation stabilized by intramolecular hydrogen bonds. Most of the oxygens lie on the inside of the complex, leaving a hydrophobic outer surface. The five oxygens O_4, O_5, O_6, O_7, and O_8 bind the cation in all six of the published structures. A carboxylate oxygen is bound to the cation in only two structures, and in no case is O_1 involved in cation binding.

In the *solution* phase there are a number of instances where the pattern of ligation clearly differs from that found in the solid state. These are summarized in a recent paper[10] from this laboratory in which we demonstrate that the specific LAS oxygens used for cation binding are determined by both the solvent polarity and the cation charge. Another recent study[11] involving Ca^{2+} and La^{3+} complexes of LAS indicates that in chloroform solution a dynamic, intramolecular ligand donor exchange occurs in which at least two ligands "take turns" binding *via* O_4, O_6, and O_7 while remaining attached to the cation at O_3 and O_8. In both these studies, crystalline complexes having the general stoichiometry $M^{n+}(LAS)_n \cdot X\ CHCl_3$ were prepared and characterized for M = Ca^{2+}, Mn^{2+}, Gd^{3+}, and La^{3+}. Here X is an integer or half-integer.

Lasalocid A is also known for its ability to bind and transport amine cations. Most of the previous work has involved biogenic amines, and the existence of 1:1 complexes in solution and their role in membrane transport is well established.[2-4,12-14] In 1977, Westley *et al.*[15] first reported the isolation and characterization of *crystalline* 1:1 LAS-amine complexes. A number of amines were used including several chiral ones for which resolution of the racemic amine occurred as the result of preferential crystallization of one diastereomer of the complex. An x-ray crystallographic study[15] of the LAS-cation complex of R-(+)-1-amino-1-(4-bromophenyl)ethane showed that the amine cation binds LAS *via* hydrogen bonds from $-NH_3^+$ to O_3, O_6, and O_8. The overall conformation of the LAS backbone in this complex was found to be the same as that in LAS complexes of metal cations. However, unlike the dimeric Na^+ and Ag^+ complexes, the amine complex is monomeric.

As an extension of our work concerned with the ligating properties of LAS toward inorganic cations in the solution phase, it seemed appropriate to attempt to establish how the lasalocid anion binds organic amine cations in the solution phase. Thus we have prepared and characterized a series of complexes of LAS having the general stoichiometry $(R\text{-}NH_3)(LAS)$, where the R-group is varied systematically over a range of organic substituents varying in steric properties and functional groups.

RESULTS AND DISCUSSION

The procedure used for preparing LAS complexes described in this paper and in previous reports from this laboratory[10,11] is designed to ensure saturation of the ionophore. In all cases, elemental analyses are consistent with the 1:1 stoichiometry (cation)(LAS). In some cases a stoichiometric amount of water is associated with the complex. Results of elemental analyses and melting points of the complexes are given in Table I. Effective molecular weights in chloroform solution are shown in Table II. The complexes are very soluble in organic solvents of low polarity such as chloroform and methylene chloride and have moderate solubilities in cyclohexane and carbon tetrachloride.

Table I. Characterization of $(Cation^{n+})(LAS)_n \cdot S$ Complexes

Complex	S	Cation	M.P. (C°)	%C calcd.	%C found	%H calcd.	%H found	%N calcd.	%N found
1		NH_4^+	170-172(d)	67.18	66.72	9.45	9.25	2.30	1.97
2	$\frac{1}{2}H_2O$	$CH_3\text{-}NH_3^+$	187-189	66.64	66.50	9.59	9.84	2.22	2.19
3		$C_2H_5\text{-}NH_3^+$	202-204	68.00	67.61	9.67	9.60	2.20	2.15
4		$t\text{-}C_4H_9\text{-}NH_3^+$	196-198	68.74	68.86	9.87	10.10	2.11	2.07
5	$\frac{1}{2}H_2O$	$C_6H_5\text{-}NH_3^+$	158-160	69.33	69.05	9.02	9.10	2.02	1.95
6		1-adamantyl-amine $\cdot H^+$	201-203	71.22	71.00	9.64	9.56	1.89	1.84
7		R-(+)-α-methyl benzylamine$\cdot H^+$	196-198 (lit.[15] 198-199)	70.85	70.99	9.20	9.48	1.97	1.80
8	$\frac{1}{2}H_2O$	S-(-)-α-methyl benzylamine$\cdot H^+$	112	69.97	70.17	9.23	9.50	1.94	1.70
9		ethyl-L-alan-inate $\cdot H^+$	158-159	66.17	66.00	9.25	9.50	1.98	1.68
10		dopamine $\cdot H^+$	216-218 (lit.[15] 220)	67.80	67.48	8.81	9.00	1.88	1.81
11		$NH_3^+\text{-}(CH_2)_{12}\text{-}NH_3^+$	189-191	69.53	69.40	9.92	10.01	2.03	1.82

Table II. Effective Molecular Weights of Selected Lasalocid A Complexes in Chloroform Solution

Complex	calcd.	found
NaLAS	613	591
1	608	620
3	636	622
4	664	652
6	742	726
8	712	700
10	744	732

Organic amines with diverse steric and electronic properties were chosen in order to assess the coordinating ability of the ionophore. Crystalline complexes were readily obtained with all primary amines, but we were unable to isolate a complex of LAS with the secondary amine R-(-)-epinephrine by the usual procedure. Stoichiometric complexes were readily obtained even with amines having large R-groups such as t-butyl and 1-adamantyl. The existence of monomeric, 1:1 complexes in chloroform solution is demonstrated by the results of molecular weight determinations (Table II). Complexes of both enantiomers of the chiral amine, α-methylbenzylamine were easily prepared. These have very similar ^{13}C nmr chemical shifts except for C_{13} and C_{19} (Table III). The long-chain diamine, 1,12-diaminododecane, apparently binds two LAS anions, one at each end. No unusual stoichiometries or structures (as assessed by molecular weight and/or ^{13}C nmr data) are found for complexes 9 and 10 in which the cation has functional groups capable of forming additional hydrogen bonds.

Table III. Carbon-13 NMR Chemical Shifts for Lasalocid A Complexes[a]

Complex	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{18}	C_{19}	C_{22}
HLAS	173.4	111.3	161.8	124.4	135.2	121.5	144.3	72.7	49.1	214.6	55.3	84.1	86.4	70.9	73.2
NaLAS	176.5	118.1	160.8	123.0	131.3	119.9	143.3	70.4	48.6	218.5	55.6	82.7	87.2	68.3	71.0
T&LAS[b]	175.4	116.0	161.3	123.2	131.7	119.7	143.7	70.5	48.2	216.2	56.4	84.7	87.7	69.7	71.6
1	175.9	116.2	161.5	123.1	131.9	119.9	143.9	70.4	49.0	217.3	56.0	82.8	87.2	70.0	71.5
2	176.1	116.5	161.7	123.3	131.9	119.9	144.1	70.5	49.0	218.2	56.2	83.1	87.6	69.6	71.2
3	175.9	116.1	162.0	123.4	131.9	119.9	144.3	70.4	49.2	218.3	56.4	83.0	87.7	70.1	71.0
4	175.7	116.2	161.9	123.4	131.8	119.8	144.4	70.5	48.9	218.2	56.3	83.2	87.2	70.0	70.9
5	175.9	115.4	161.9	123.6	132.5	120.2	144.5	70.5	49.2	217.8	56.2	83.5	87.7	70.4	71.5
6	175.7	116.4	162.0	123.4	131.8	119.7	144.5	70.7	48.9	217.8	56.3	83.2	87.3	70.1	70.7
7	175.9	116.0	162.3	123.6	132.2	120.1	144.6	70.6	49.3	218.4	56.5	83.1	87.7	70.3	71.1
8	175.8	116.0	162.2	123.5	132.1	119.9	144.5	70.4	49.5	219.2	56.6	83.0	87.5	68.9	71.0
9	175.9	116.1	162.1	123.6	132.2	120.0	144.5	70.7	49.4	218.7	56.7	83.1	87.7	70.1	71.2
10	176.2	115.8	161.4	123.6	132.4	120.3	144.8	70.8	48.6	218.0	56.2	83.4	87.9	70.1	71.5
11	176.0	116.2	162.1	123.5	132.0	120.0	144.4	70.5	49.2	218.2	56.5	83.1	87.7	70.3	71.1

[a] In ppm from Me_4Si. Data from chloroform-d solutions.

[b] Data from reference 19.

Infrared spectra of NaLAS and of complex 3, formed from the cation of one of the more reactive amines, $CH_3CH_2NH_2$, were compared in order to test for the occurrence of Schiff base or amide reaction products in 3. The spectra are virtually identical. In the 1000-2000 cm^{-1} region, absorbances occur at the following frequencies (in cm^{-1}) for 3 and NaLAS, respectively: 1022(w), 1020(w); 1048(s), 1048(s); 1103(s), 1103(s); 1248(w), 1254(w); 1281(s), 1275(s); 1321(s), 1321(s);

1385(vs), 1383(vs); 1429(vs), 1429(s); 1456(vs), 1458(vs); 1593(vs), 1597(vs); 1707(s), 1705(s). Thus there is no evidence for covalent bond formation between the amine and LAS.

Binding Sites of Amine Cations. Crystallography has shown that the cation of R(+)-1-amino-1-(4-bromophenyl)ethane binds LAS via hydrogen bonds to O_3, O_6, and O_8.[15] Other amine cations may also bind LAS in this manner in the solid state, but as pointed out in the Introduction, the same LAS oxygens are not necessarily used for hydrogen bonding in solution.

In an effort to establish the nature of the amine-LAS interaction in the solution phase, ^{13}C nmr spectra of the complexes in chloroform solution were examined. Proton nmr spectra of LAS complexes on the Bruker WP-80 spectrometer (80 MHz) are insufficiently resolved for this purpose. Chemical shifts of readily-observed ^{13}C signals having firm assignments[7,16-18] are given in Table III. Spectra of the amine cation complexes are very similar to that of NaLAS except for superposition of signals arising from the amine substituent (see Fig. 1). The latter signals were assigned with the aid of routine off-resonance decoupling procedures but are not listed in Table III. Chemical shifts for HLAS(lasalocid acid), NaLAS, and TlLAS are also given for comparison.

It is readily seen from Table III that chemical shifts observed for the amine complexes are generally closer to those of the Na^+ and Tl^+ complexes than to those of HLAS, indicating that LAS complexes of amine cations and monovalent metal cations probably have very similar structures in solution. Virtually nothing is definitely known regarding the binding sites for Na^+ in the solution phase. However, the ^{13}C nmr spectrum of TlLAS in chloroform solution at low temperatures shows a pattern of signal splitting, due to $^{203,205}Tl$-^{13}C coupling, which strongly indicates Tl^+ binding at O_3, O_5, O_6, and O_8.[19] There is no evidence of Tl^+ binding at O_1, O_4, and O_7. This provides a starting point for interpretation of the nmr data of the amine complexes. The ionic radii of Tl^+ and NH_4^+ are similar (1.54 A and 1.66 A, respectively[20]), and it might be expected, a priori that they would seek the same LAS binding sites and induce similar conformations in the cation-bound ionophore.

Chemical shifts of the Tl^+ and NH_4^+ complexes differ by 0.5 ppm or less for all carbons except C_{12}, C_{13}, and C_{15}. This implies that these ions bind LAS in a similar manner with the possible exception of oxygens near C_{12}, C_{13}, and C_{15} (O_5 and O_6). The smaller differences in chemical shifts for C_1, C_2, C_3, C_{11}, C_{19}, and C_{22} indicate that, like Tl^+, the NH_4^+ cation binds LAS at O_3 and O_8 but not at O_1, O_4, or O_7. (The C_{23} signal is obscured by the solvent resonance.) Larger chemical shift differences observed for C_{12} and C_{13} suggest that, unlike Tl^+, NH_4^+ is not bound to O_5. Similarly, the chemical shift difference for C_{15} implies that Tl^+ and NH_4^+ interact differently with O_6. However, this latter implication is questionable in view of (a) the relatively small chemical shift difference at C_{18}, (b) the unexplained large differences in Tl-C coupling constants between C_{15} and C_{18},[19] and (c) the unexpectedly large differences in spin-lattice relaxation rates of C_{15} and C_{18} induced by paramagnetic ions bound at O_6.[10,21] Overall, the ^{13}C nmr data indicate that NH_4^+ binds at O_3, O_8, and probably O_6. It cannot be ascertained whether NH_4^+ forms a fourth hydrogen bond.

Chemical shifts among the other amine complexes are rather similar and in general differ only slightly from those of NH_4LAS (largest difference is at C_{13} for 8). Some differences might be expected for the NH_4^+ complex, since this is the only cation capable of forming four hydrogen bonds. Among complexes 2-11, the observed chemical shift differences are attributed to steric effects of the R-group, aromatic ring currents, and other electronic influences of the R-group. Complex 7 is of particular interest, since the cation is closely related to the R(+)-1-amino-1-(4-bromophenyl)ethane cation which has been shown to bind LAS at O_3, O_6, and O_8 in the solid state.[15] Chemical shifts for 7 show no unusual deviations from those of the other complexes. If it is assumed that the R-(+)-α-methylbenzyl ammonium cation binds LAS in chloroform solution in the same manner as its (4-phenyl) brominated analog in the solid state, the nmr data imply binding at O_3, O_6, and O_8 for all the amine cation complexes.

The carbon-13 nmr spectrum of $(C_2H_5NH_3)$(LAS), 3, was also examined in N,N-dimethylformamide (DMF) solution to test whether solvent polarity effects on ligation, such as those observed for metal cations[10], could be discerned. On going from chloroform to DMF solutions, chemical shift changes of 0.2-1.4 ppm were observed for most carbons of 3. Similar changes are observed also with

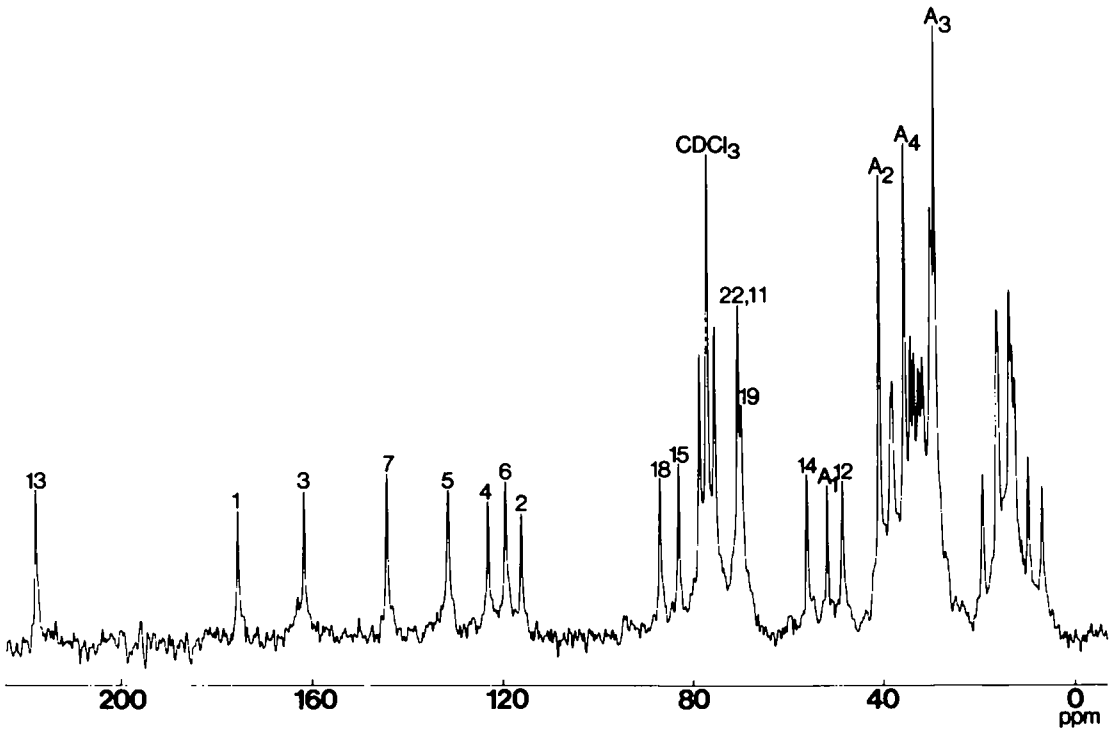


Figure 1. 20-MHz carbon-13 nmr spectrum of complex 6, (1-adamantylamine·H)(LAS), in chloroform solution. Signals arising from the adamantyl group are assigned as follows: A_1, C_1(52.1 ppm); A_2, C_2 + C_8 + C_9 (41.1 ppm); A_3, C_3 + C_5 + C_7 (29.2 ppm); A_4, C_4 + C_6 + C_{10} (35.8 ppm).

NaLAS[10] and do not necessarily result from changes in ligation. However, the chemical shift change of 2.3 ppm found for C_{22} of 3 is exceptional (the corresponding change is only 0.6 ppm for NaLAS) and may indicate O_8 does not bind the amine in the more polar solvent. A similar situation was found for the Mn^{2+} cation[10].

Summary. Crystalline, 1:1 complexes of the lasalocid A anion with cations of a variety of primary amines are readily prepared. IR spectra show that the amines do not react with lasalocid A other than to form hydrogen bonds. Molecular weight data demonstrate that in chloroform solution the ions remain closely associated. Carbon-13 nmr data in chloroform solution indicate that O_3, O_8, and probably O_6 of the lasalocid A anion are involved in hydrogen bonding to the cations as has been found for a related complex in the solid state.

EXPERIMENTAL

Materials. NaLAS was purchased from Aldrich Chemical Company and used without further purification, since ^{13}C nmr chemical shifts were in good agreement with reported values,[7,16] and no signals attributable to impurities could be detected.

Some of the amines used are available as their hydrochloride salts and, with the exception of aniline·HCl, were used without further purification. Aniline·HCl was recrystallized several times using methanol and ethyl ether. The hydrochloride salts of other amines, with the exception of R-(-)-epinephrine, were prepared by passing HCl through a solution of the amine in ethyl ether. The resulting precipitates were washed several times with ethyl ether then dried in vacuo. R-(-)epinephrine·HCl was prepared by acidifying an aqueous solution of the amine followed by removal of the solvent in vacuo.

Preparation of LAS Complexes. The procedure used to prepare LAS complexes of amine cations was a slight modification of that reported previously for preparation of LAS complexes of metal cations.[10,11] In a typical preparation, a 30 ml aqueous solution containing 10 mmol of the amine hydrochloride is divided into three 10-ml portions. Each portion, in turn, is stirred vigorously for 2-3 hr with 25 ml of a chloroform or methylene chloride solution containing 1 mmol NaLAS. The non-aqueous layer is then washed several times with water and dried over molecular sieves. Subsequent filtration followed by removal of the solvent in vacuo affords a crystalline product.

Instrumentation. Carbon-13 nmr spectra were obtained on a Bruker WP-80 FT spectrometer operating at 20 MHz. Spectra were run at the ambient probe temperature of 39°C using 8K data points. Elemental analyses were carried out on a Hewlett Packard Model 185B C, H, N analyzer located in the Department of Medicinal Chemistry. Repeated analyses of the same sample on this instrument spanned a range of 0.5% for C and 0.4% for H and N. Nitrogen analyses were usually low. Molecular weight determinations were made in chloroform solution at 37°C using a Hewlett Packard Model 302 vapor pressure osmometer. The osmometer was calibrated using recrystallized benzil, and solutions were in the concentration range of 1 to 10 mmolar. Infrared spectra were run on KBr pellets using an IBM Instruments IR 32 FT spectrophotometer. All melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected.

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