Open and Closed Forms of the Ionophore Lasalocid Free Acid and Free Anion. Obtaining the Most Probable Conformations Using AM1 Semi-empirical Quantum Mechanical Calculations

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Open and closed conformations of both lasalocid free acid and lasalocid free anion are obtained through optimization processes using the AM1 program. These results are compared with structural information on the solid state and solutions in various solvents. Attention is focused on the opening–closure process of the ionophore.

Lasalocid A (Fig. 1), an antibiotic of the carboxylic polyether series, is well known for its ability to transport metal cations across natural and artificial membranes. The complexing–decomplexing process at the membrane–solution interface is governed by an opening–closure process of the shell formed through interaction of the carboxy and hydroxy groups located at the two ends of the molecule. Knowledge of the conformations of the ionophore in its two limit-states, open and closed, and of the energies associated with this conformational change are thus of prime importance. Some insight on the conformation of lasalocid acid and its anion in solution was provided by NMR studies. Complete and unequivocal assignments of the proton and carbon resonance shifts of free lasalocid acid and anion in chloroform and methanol were recently performed;[1] most of the ^1H–^1H coupling constants were also accessed. From this data and the previous work of Painter[2] on ^{13}C-relaxation, a picture of lasalocid structures in the two solvents emerges. Contrary to other lasalocid species, the acid form of lasalocid is monomeric in both methanol and chloroform. In chloroform, lasalocid occurs in a globular form, hydrogen bonding between the terminal hydroxy and one of the carboxy oxygens ensuring the closure of the pseudo-ring resulting in a relatively constricted structure. In methanol, marked freedom of the salicylic arm relative to the rest of the backbone is observed; increased rotational freedom around the C(8)–C(9) bond corresponding to fast interconversion between rotamers facilitates the opening of the pseudoring. This is not so for the anion form for which the rotation of this bond was shown[1] to be severely hindered probably through interactions of the carboxylate with the hydroxy group on C(11).

Structures of lasalocid and bromolasalocid crystals were previously determined[3–5] from X-ray data. One is a dimer, the other monomers. All of them contain one molecule of water, methanol or ethanol. In the monomeric forms, lasalocid is folded by head-to-tail hydrogen bonding. A molecule of crystallization solvent retained and bound to some of the oxygens or hydroxy protons of lasalocid is involved in this folding and closure. Of cations, only tetraalkylammonium ions are not complexed by lasalocid anion. Their lasalocid salts occur in solution either as free ions in polar solvents or as ion pairs or multiplets in apolar media.[1] No crystals of such salts have been isolated yet, and so references for the free anion structure can only be inferred from theoretical computations; a first computer model of lasalocid anion using consistent force-field calculations was provided by Painter *et al.*[6]

To investigate more thoroughly the opening–closure process, a theoretical search for most favoured conformations of lasalocid free acid in its open and closed structures was undertaken. This was conducted by AM1, a quantum semi-empirical method, proposed by Dewar.[7] In addition, to compare data, some of the calculations were also processed using the consistent force-field MM2 program proposed by Allinger.[8] As a first step, to shorten calculation times, interactions with solvent molecules were not considered. For the closed structure, calculations were started using initial coordinates resulting from the X-ray analysis of a lasalocid silver salt crystal.[9] These calculations were expected to provide a conformational description of the most stable closed structure, together with information on its internal hydrogen bonding system.

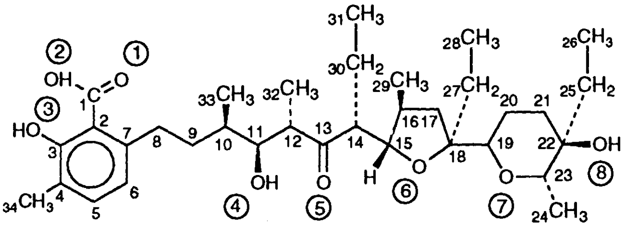

Fig. 1 Lasalocid formula showing the numbering system used for carbon and oxygen atoms

Smooth opening of such a structure could most probably be achieved by rotating the C(8)–C(9) or the C(18)–C(19) bonds. C(12)–C(13) bond rotations were also investigated. A systematic investigation of conformations optimized for various values of the corresponding dihedral angles was thus undertaken. Most stable open conformations and corresponding rotational barriers could thereby be obtained. Starting from the various conformations retained for the lasalocid free acid a search could be made for the most favourable open and closed conformations of the anion.

Finally, a purely empirical full unfolding of the lasalocid backbone was carried out to obtain conformations analogous to that suspected for the lasalocid anion in its monomer and aggregates in water.[10]

Experimental

The program used was AM1 in MOPAC v 6.10,[11] all the geometries being optimized by energy minimization (BFGS method). Some research was also conducted using the MM2[12] consistent force-field program. Calculations were achieved using the Vax 6410 computer at the Computational Center at Blaise Pascal University.

Table 1 Energy and conformational parameters for some of the free acid computed geometries [formation enthalpies $\Delta_f H$, entropies S, hydrogen bond systems, tetrahydrofuran (THF) and tetrahydropyran (THP) cycle conformations and dihedral angle θ between the benzene and the carboxy group planes]

	I	I′	III	IV	VI
$\Delta_f H$/kcal mol^{-1}	−417.5	−420.2	−415.5	−415.6	−419.4
S/cal K^{-1} mol^{-1}	264.7	270.7	270.6	267.1	
Hydrogen bond length/Å					
O(2)–H ··· O(4)	2.15			2.29	
O(2)–H ··· O(8)		2.07			
O(3)–H ··· O(1)	2.12	1.98	2.03	2.06	1.98
O(4)–H ··· O(2)		2.39			
O(4)–H ··· O(6)	2.45		2.24	2.31	
O(4)–H ··· O(8)	2.34				
O(8)–H ··· O(1)	2.22				
O(8)–H ··· O(4)	2.43		2.58		
O(8)–H ··· O(7)		2.39	2.41		2.44
Conformational aspects					
THF	Plan[a]	Env.[b]	Plan	Plan	Plan
THP	Chair	Chair	Chair	Chair	Chair
θ/°	−41.7	−22.7	−31.6	−34.9	−20.7

[a] Plan = planar. [b] Env. = envelope.

Methods and Results

No systematic search, for covering the whole conformational space by rotating all the C–C bonds of the backbone was undertaken. It was clear that with such a molecule it would have generated a very large number of conformations. Though smaller, still appreciable number of conformers could also be obtained by Monte-Carlo mechanics calculations. For example, calculations, undertaken after completion of the work reported here, and using the 'Batchmin' program coupled with the MM2 or the MM3 force fields (Macromodel package)[13] allowed us to generate 164 and 706 respectively, different stable geometries in a 3 kcal mol^{-1} margin.* Here, in order to limit our investigations the choice of the initial conformers and the selection of the rotatable bonds was done in strong conjunction with the known experimental results: X-rays, structures, studies in the solid state, NMR data (chemical shifts, coupling constants, NOE and ^{13}C relaxation times) as well as other spectroscopic and thermodynamic data in solution.

Though the numbering system used in the computations differs for oxygen and hydrogen atoms from the system shown in Fig. 1, for consistency with previous and forthcoming papers in these series, only this last system will be used for reporting present data. In this system, geminal hydrogens are referred to as A and B according to the location of their ^{1}H NMR chemical shift (A at lower field). In dubious cases, unambiguous identification between the two systems was achieved by comparing, *a posteriori*, calculated with experimental ^{1}H–^{1}H coupling constants in closed forms.

Optimizing the Structure of the Lasalocid Free Acid Closed Form.—A starting geometry of the lasalocid free acid was obtained using absolute coordinates of a closed form of the lasalocid anion in a silver salt crystal,[9] and then adding corresponding necessary hydrogens on carbon and oxygen atoms. In such a crystal, as in some other lasalocid structures, there are two alternative positions of the terminal ethyl group corresponding to schemes *a* and *b* in Fig. 2; coordinates corresponding to form B were chosen here as a basis for calculations performed using AM1. After many optimization cycles, conformation I was generated. As shown in Fig. 3 it is a

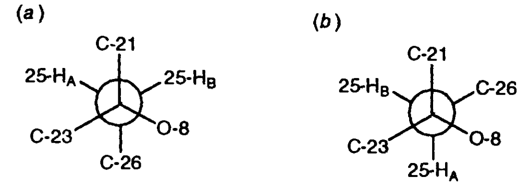

Fig. 2 Alternative positions, *a* and *b*, of the terminal ethyl group in lasalocid

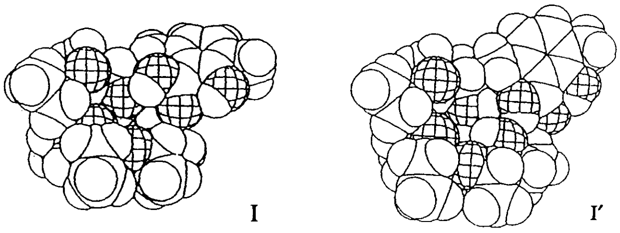

Fig. 3 Compact modelling of lasalocid free acid closed forms I and I′; ⊕ oxygens, ○ carbons, ∘ hydrogens

rather compact structure. Other characteristic features of lasalocid acid geometry I are presented in the first column of Table 1, in Fig. 4 I and in Fig. 5. In Fig. 5 conformational aspects of the lasalocid backbone and particularly the geometry of the cycle are stated. Quantitative specification of the ring puckering was done using Cremer and Pople definitions.[14] z Distances of the cycle vertices to a mean plane defined by $\Sigma z_j = 0$ are considered. For a five-membered ring these distances are related to parameters q (maximum value of z_j) and φ (phase angle) by the formula shown in eqn. (1) with φ respectively equal to 36°

$$z_j = (2/5)^{\frac{1}{2}}\, q \cos\,[\varphi + 4\pi(j - 1)/5] \qquad (1)$$

and $(36\,p + 18)°$ for envelope and twist conformations. For a six-membered ring z_j distances are related to three parameters according to eqn. (2): θ takes values of 0 and 180° for the chair

$$z_j = 3^{-\frac{1}{2}}\, q\{\sin\theta \cos\,[\varphi + 2\pi(j - 1)/3] + [(-1)^{j-1}\, 6^{-\frac{1}{2}} \cos\theta]\} \qquad (2)$$

form, 90° for the flexible forms and the phase angle φ is thus

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* 1 kcal = 4.184 kJ.

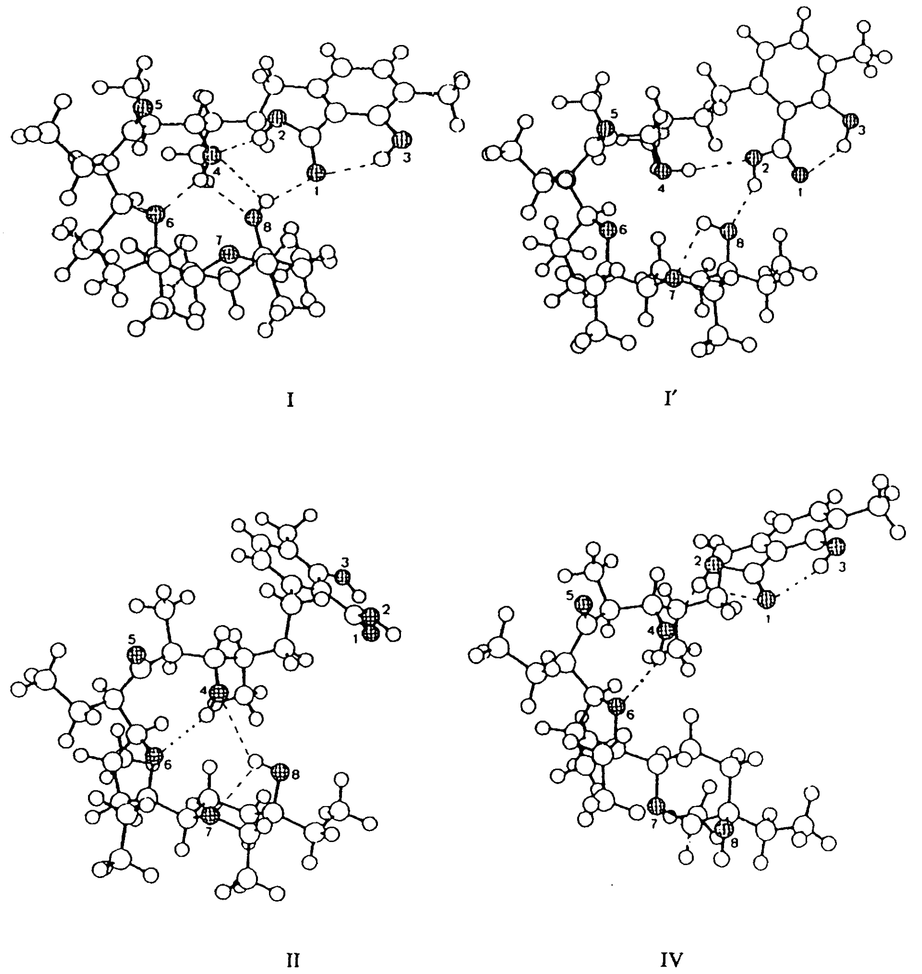


Fig. 4 Skeleton schemes of some of the more stable closed and open computed geometries of the lasalocid free acid showing their hydrogen bonding system; ◯ carbons, ○ hydrogens, ⊕oxygens

respectively 2 *p* (30)° and (2 *p* + 1) (30)° for boat and twist conformations. Concerning lasalocid acid geometry **I**, processing of the data suggests a near-flat conformation for the tetrahydrofuran cycle and a chair conformation for the tetrahydropyran cycle (see Fig. 5).

Form A of geometry **I** (see Fig. 2) was also generated by rotating by 60° around the C(24)–C(25) bond. This resulted, after optimization using AM1, in a quasi-identical structure except for this single difference. The enthalpy of formation was a little lower: −417.8 against −417.5 kcal mol^{-1}. However form B, already used as a basis for generating other forms, was retained.

Another stable acid form was generated starting from an anionic geometry obtained, as described further on, by rotating around the C(12)–C(13) bond. This form **I'** differs from form **I** mainly by closure with an O(8)–H ··· O(2) hydrogen bond instead of an O(8)H ··· O(1) hydrogen bond, O(1) being the one oxygen of the carboxylate group already bound to the phenolic oxygen O(3). This closure change results in an appreciable modification of the hydrogen bond system as shown in Table 1 and in slight modifications to the chain dihedral angles, at most 20°, stronger variations being observed for dihedral angles involving O(2)H and O(4)H hydroxy groups.

Opening the Structure of the Lasalocid Free Acid by Rotating the Two Terminal Moieties Around the C(8)–C(9) *and* C(18)–C(19) *Bonds.*

—Starting from closed conformation **I**, C(1)–C(8)–C(9)–C(10) and C(17)–C(18)–C(19)–C(20), dihedral angles were independently rotated from −180 to +180° in 30° steps, structural optimization of all the other coordinates being achieved by the AM1 program. Enthalpy of formation minima were obtained by re-optimizing the whole geometry. Results were also obtained using the MM2 program, proceeding in smaller steps. Both sets of data are reported in Fig. 6. Those using AM1 are more regular and may be considered as more reliable, but except for differences in absolute values of the formation enthalpies, the MM2 force-field method gives results consistent with the AM1 method.

Rotating around the C(8)–C(9) bond generated two other stable geometries, **II** and **III**. Their molar enthalpies of formation would differ only by 2 kcal (conformation **III**) and 3 kcal (conformation **II**) from that of conformer I, enthalpy barriers being of the order of 2.9 and 4.6 kcal respectively. Rotating around the C(18)–C(19) bond also generated two other stable forms with molal formation enthalpies higher than that of form **I** by 1.8 kcal (**IV**) and 2.4 kcal (**V**), energy barriers being respectively 4.3 and 6.4 kcal. These four conformations (**II–V**) are opened through disruption of the O(8)–H ··· O(1) hydrogen bond, their whole internal hydrogen bond system being consequently modified, differently for each one, as shown by distances reported in Table 1. Conformations of the two cycles are analogous to that reported for geometry **I**, except for the tetrahydrofuran cycle in geometry **IV**. In this form a

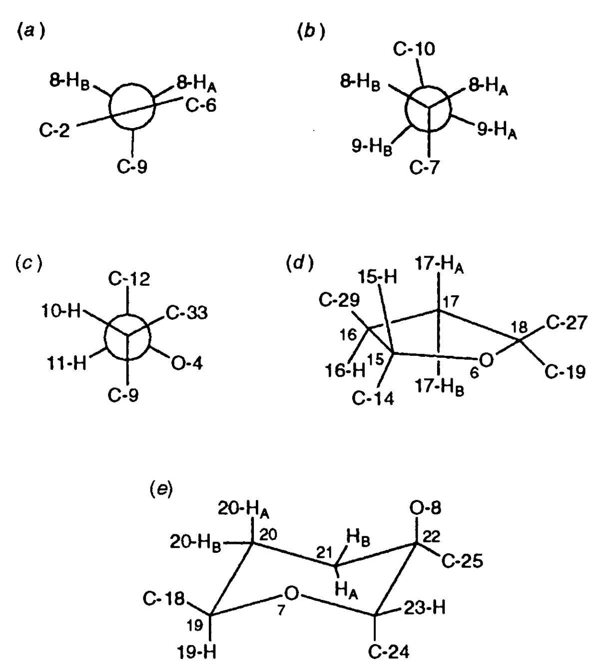


Fig. 5 Some schematic details of conformation of lasalocid free acid geometry **I**. Newman projections along the (*a*) C(7)–C(8), (*b*) C(8)–C(9) and (*c*) C(10)–C(11) bonds, and conformations of the (*d*) tetrahydrofuran and (*e*) tetrahydropyran rings.

departure of this cycle from its near-flat structure is observed; a twist form is generated.

The dihedral angles between the benzene ring plane and the carboxy group plane are also given in Table 1. This angle is never zero, even in the open geometries, though smaller there, and varies appreciably, from 42° in form **I** to 21° in form **II** for example. Absolute entropies for the various geometries, calculated by the THERMO subroutine in the AM1 program, assuming no internal rotation, are also reported in Table 1. As expected, this entropy is greater for open than for closed structures.

Most Probable Open and Closed Structures of the Lasalocid Anion.—Anion geometries were generated using two procedures; first starting from the stable geometries obtained from the free acid, withdrawing the carboxylic proton and adding a minus charge; second, starting from closed form $\mathbf{I}_a$ thus obtained and rotating around the C(12)–C(13) bond. Optimized geometries of the anion obtained by the first procedure are labelled with subscript a, according to their parent acid geometries.

The second procedure generated a stable form closed through an O(8)H ··· O(2) hydrogen bond, $\mathbf{I'}_a$. The most significant results are reported in Table 2 and Fig. 7. Compared with the corresponding acid forms the dihedral angle variations are weak, though sometimes significant; a reinforcement of the subsisting hydrogen bonds is generally observed.

Fully Unfolded Structure.—To determine how the lasalocid molecule could be unfolded all the dihedral angles of the carbon backbone were *a priori* taken as 180°. The optimizing procedure then generated a geometry designated **VI** the parameters of which are reported in the last column of Table 1. Surprisingly, the formation enthalpy of lasalocid in this conformation is lower than in conformations **I** and **I'**. A corresponding geometry for the lasalocid, anion $\mathbf{VI}_a$, was thus obtained by withdrawing the carboxylic proton and using AM1 computations as previously. Some of the results obtained are shown in Table 2 and Fig. 8.

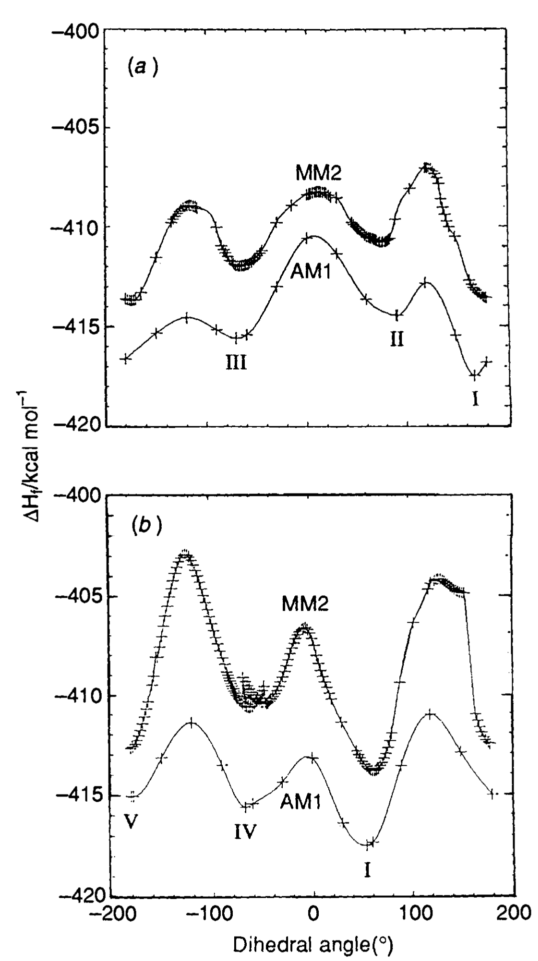
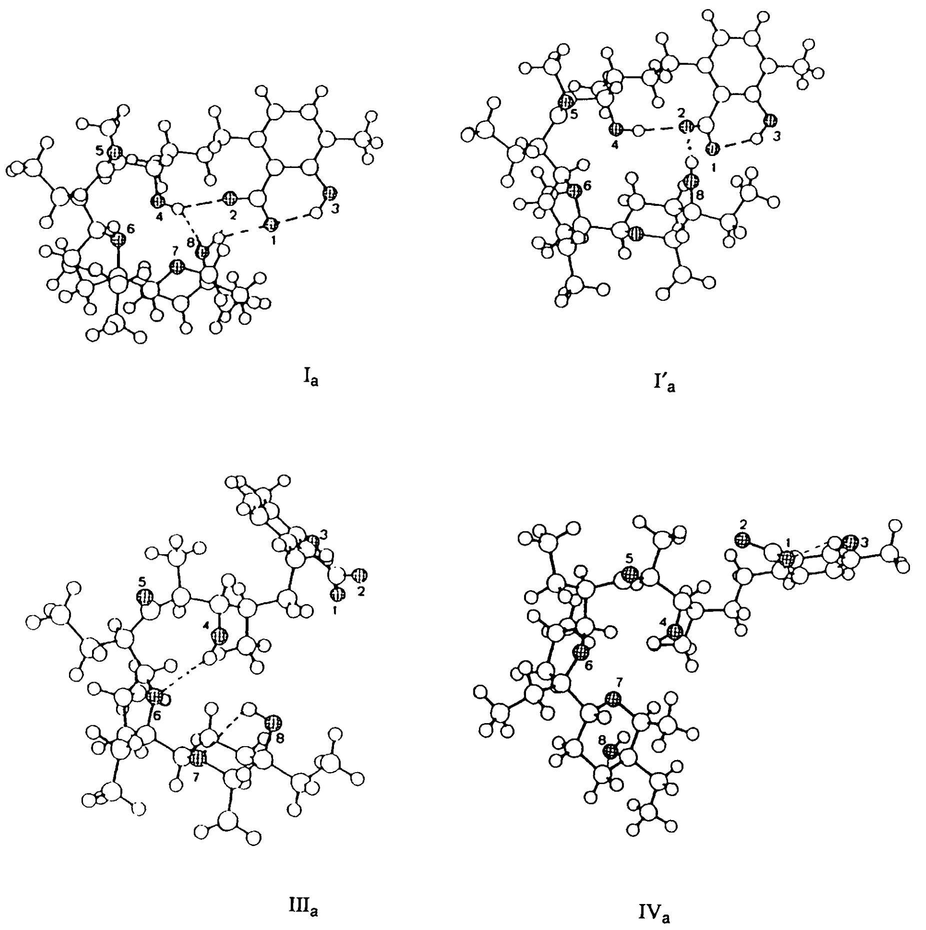


Fig. 6 Formation enthalpies of free lasalocid acid geometries generated through rotations around (*a*) the C(8)–C(9) and (*b*) the C(18)–C(19) bonds using force field MM2 and semi-empirical AM1 calculation methods

Discussion

Lasalocid Acid and Anion in Crystals.—Molecular structures were derived from X-ray studies of crystals of lasalocid and its parent compound 5-bromo- or 5-nitro-lasalocid; a solvent molecule, water, methanol or ethanol, is present in all the crystals so far investigated and is thus part of the hydrogen bonding system of the molecule. Both monomer and dimer structures were recognized. In the acid crystals [3–5] the monomer units are all closed by head-to-tail hydrogen bonding through O(2)–H ··· O(8) ligation, other hydrogen bonding being O(3)–H ··· O(1) and O(4)–H ··· O(2). This geometry is thus akin to form **I'** obtained here though slight conformational changes occur through chelation to the solvent molecule and possibly interaction between monomer units.

Concerning the lasalocid anion, no solid structure involving an uncomplexed cation (such as a tetraalkylammonium ion) has yet been isolated, though some crystalline metal cation complex salts have been obtained.[16] X-Ray investigations of these various crystals show that for monovalent metal lasalocid salts, monomeric, dimeric and exceptionally polymeric molecular structures are encountered. In these structures lasalocid anions are closed preponderantly through O(8)H ligation either to O(1), for example in the silver salt or to O(2), for example in the caesium salt. These two different types of ligation were observed even in the two molecules of a dimeric structure, for the 5-nitrolasalocid silver salt.[16] Thus in the solid state, lasalocid anion conformations in monovalent metal cation salts can be akin to both $\mathbf{I}_a$ and $\mathbf{I}_a'$ geometries computed here. Obviously,



I_a I'_a III_a IV_a

Fig. 7 Some stable closed and open computed geometries of the lasalocid anion

Table 2 Energy and conformational parameters for some of the anion computed geometries (notations analogous to Table 1)

	I_a	I_a'	III_a	IV_a	VI_a
Δ_fH/kcal mol^{-1}	−450.1	−454.8	−442.4	−439.2	−448.5
Hydrogen bond length/Å					
O(3)–H ··· O(1)	1.88	1.95	1.94	1.90	1.89
O(4)–H ··· O(2)	2.31	2.02			
O(4)–H ··· O(6)			2.41		
O(4)–H ··· O(8)	2.29				
O(8)–H ··· O(1)	2.26				
O(8)–H ··· O(2)	2.14	2.11			
O(8)–H ··· O(7)			2.49		2.44
Conformational aspects					
THF	Env.	Env.	Plan	Env.	Plan
THP	Chair	Chair	Chair	Chair	Chair
θ/°	−26.3	−38.3	−40.3	−33.8	−30.3

owing to cation coordination and subsequent disruption of the internal hydrogen bonds, conformational changes secondary to these original geometries are observed.

Lasalocid Acid and Anion in Chloroform and Acetonitrile.—Lasalocid free acid was shown to occur as a monomer in both chloroform and methanol.[1,17] This can be assumed to be the case also in acetonitrile solutions. In the aprotic solvents, at least, the lasalocid molecule is presumably closed through head-to-tail ligation, such closure being stabilized by internal hydrogen bonding, which corresponds here to geometry **I** or **I′**. Experimental ^1H–^1H coupling constants previously acquired in acetonitrile[15] and chloroform[15,17] can be compared in Table 3 to values calculated from these computed geometries. For this purpose we used extended Karplus calculations. The Durette and Horton formula[18] was preferred here since it was parametrized by means of coupling constants found in carboxylate compounds involving cyclic ethers as here. The atomic electronegativities used in the calculations were those of Huggins.[19] Comparison of experimental constants in acetonitrile and chloroform with values calculated for closed geometries **I** and **I′** are, as a whole, satisfactory. Such a general fit was not achieved using the Altona formula,[20] which confirmed that, the Durette and Horton formula is a better choice. Nevertheless,

Table 3 Vicinal ^1H–^1H coupling constants (Hz) for lasalocid free acid: experimental values in chloroform,[1] acetonitrile[15] and methanol[1] and values calculated according to Durette and Horton[18] for some of the geometries here obtained[a]

	Experimental			Computational					
	CD_3OD	CD_3CN	$CDCl_3$	**I**	**I**′	**I**″	**II**	**III**	**IV**
8A–9A		4.8	3.2	2.5	2.3	2.6	9.1	12.7	2.6
8A–9B		12.0	12.8	12.5	12.3	12.5	1.9	2.9	12.6
8B–9B		5.4	5.0	5.2	5.5	5.2	11.2	6.1	5.1
8B–9A		12.0	12.8	12.5	12.4	12.6	1.9	2.8	12.6
10–33	6.3	7.0	7.0	7.2*	7.2*	7.2*	7.2*	7.2*	7.2*
10–11	1.5	2.4	2.4	3.4	3.3	3.3	1.9	1.7	2.6
11–12	9.5	9.6	9.6	10.2	10.0	9.8	9.0	10.1	8.7
12–32	7.0	6.6	6.6	7.0*	7.0*	7.0*	7.0*	7.0*	7.0*
14–30A	9.6	10.2	10.6	11.3	11.7	10.4	11.3	11.3	11.5
14–30B	3.2	3.0	2.5	2.0	2.1	1.9	2.0	2.5	2.0
14–15	3.2	3.0	2.4	2.7	1.7	2.1	2.5	2.5	2.1
15–16	10.0	9.6	10.4	6.2	7.7	9.0	6.2	6.2	8.5
16–17A	7.5	7.7	7.7	5.2	6.9	8.9	5.6	5.7	8.9
16–17B	11.5	13.0	12.6	10.9	10.4	9.4	10.8	10.9	9.4
16–29	6.5	6.4	6.5	7.2*	7.2*	7.2*	7.2*	7.2*	7.2*
19–20A	10.5	11.4	12.0	10.9	11.1	10.9	11.1	11.0	11.2
19–20B	3.0	2.4	2.4	4.6	4.2	4.7	4.3	4.3	3.9
20A–21A				13.0	13.0	13.0	12.9	12.9	13.0
20A–21B	5.0			5.3	5.4	5.3	5.6	5.6	5.3
20B–21A	6.5			5.2	5.2	5.2	5.4	5.5	5.2
20B–21B				3.4	3.4	3.4	3.2	3.2	3.3
23–24	7.0	7.0	7.0	6.4*	6.4*	6.4*	7.2*	7.5*	6.4*
25–26	7.0	7.0	7.0	7.5*	7.5*	7.5*	7.5*	7.5*	7.5*
27–28	6.7	7.0	7.5	7.5*	7.5*	7.5*	7.5*	7.5*	7.5*
30–31	7.0	7.0	7.5	7.4*	7.5*	7.5*	7.5*	7.5*	7.5*

[a] * = Mean calculated values for methyl groups.

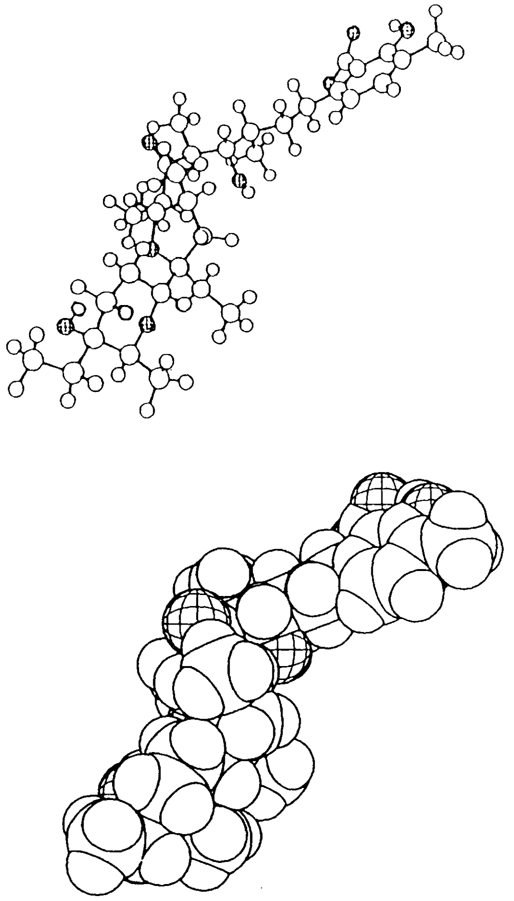


Fig. 8 An unfolded structure of the lasalocid anion

appreciable discrepancies between calculated and experimental coupling constants are observed in the furan cycle, in the C(15)–C(17) area.

Starting with conformation **I**, we tried to set the C(14)–C(15)–C(16)–C(17) dihedral angle to have a J_{15-16} coupling constant of the order of 10, as observed experimentally; acceptable values of this dihedral angle were then −160, +160 or 0. The Sybyl molecular modelling Package[21] was used; this constraint was maintained during optimization by the included Tripos forcefield. The resulting geometry was thus reoptimized by AM1 without any constraint. Optimization finally resulted in the usual geometry **I** except in the last case for which a structure **I**″ slightly different from **I**′ was obtained. The tetrahydrofuran cycle is here in an envelope form, the apex being C(16). The corresponding ^1H–^1H coupling constants are also reported in Table 3. General agreement with experimental coupling constants is not better than for geometries **I** and **I**′; a better fit is obtained for J_{15-16}, a worse one for J_{16-17A} and J_{16-17B}.

The anion obtained by dissolving any tetraalkylammonium salt of lasalocid in the aprotic solvents chloroform or acetonitrile, was now considered. The anion was shown to be more or less engaged with the cation in an ion pair but definitely not in a complex.[1] Structurally it can thus be considered as a free anion. The loss of the proton and the creation of a minus charge in the carboxylic group reinforce the head-to-tail ligation[1] *i.e.* the hydrogen bond between O(8) and either O(1) or O(2) or both O(1) and O(2). Computed and experimental coupling constants are reported in Table 4. Interestingly, experimental data in acetonitrile agreed well with the computed geometry $\mathbf{I}_a'$, unlike experimental constants in chloroform, where discrepancies with the two computed geometries, either $\mathbf{I}_a$ or $\mathbf{I}_a'$ were more marked on average.

Lasalocid Acid and Anion in Methanol.—A thorough examination of the ^{13}C longitudinal relaxation time of all the carbons of the acid molecule in methanol was made by Painter.[2] He showed that the portion of the molecule from C(10) to C(23) was fairly rigid but that the salicylic moiety was, as a whole, more mobile. In addition, chemical equivalence between protons 8A and 8B more precisely suggested rotation around the C(8)–C(9) bond and fast interconversion between rotamers.[1]

Table 4 Vicinal ^1H–^1H coupling constants (Hz) for lasalocid anion: experimental values in chloroform,[1] acetonitrile[15] and methanol[1] and values calculated according to Durette and Horton[18] for some of the geometries here obtained[a]

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8A–9B	11.2	12.0	12.5	12.8	12.9	4.7	12.4
8B–9B	5.6	5.5	5.6	4.4	3.8	4.8	5.3
8B–9A	11.2	12.0	12.5	12.8	12.9	3.8	12.6
10–33	6.5	6.3	7.0	7.1*	7.2*	7.2*	7.2*
10–11	1.8	2.6	1.2	2.7	3.8	1.6	3.1
11–12	10.0	9.8	10.0	9.0	9.8	8.6	9.8
12–32	7.0	7.0	7.0	7.0*	7.0*	7.0*	7.0*
14–30A	10.0	9.8	10.0	11.7	11.8	11.4	12.2
14–30B	4.0	3.0	2.5	2.1	2.2	2.0	2.8
14–15	4.0	1.5	2.5	2.1	1.6	2.1	10.3
15–16	10.0	9.8	10.4	6.8	9.0	6.6	4.6
16–17A	8.0	7.5	5.0	4.6	8.5	6.1	5.5
16–17B	11.0	12.5	10.5	10.9	9.7	10.7	10.9
16–29	6.5	6.3	6.5	7.2*	7.2*	7.2*	7.2*
19–20A	10.0	11.3	11.2	11.2	11.2	11.0	11.1
19–20B	3.2	3.0	1.6	3.2	3.5	4.3	4.1
20A–21A	11		9.0	13.2	13.2	12.8	12.9
20A–21B	5		3	4.2	4.9	5.8	5.6
20B–21A	6	4	4.5	4.2	4.8	5.6	5.5
20B–21B				4.1	3.7	3.1	3.2

[a] * = Mean calculated values for methyl groups.

The present computation shows that such a rotation is easy: starting with a closed conformation like geometry **I**, the enthalpies of the generated stable conformers, **II** and **III**, are slightly higher but their entropies (Table 3) partially compensate so that the free energy difference between, for example, geometries **I** and **III** is low, 0.9 kcal mol^{-1}. The enthalpic barriers to rotation are weak (Fig. 6) and they can possibly be lowered through solvation by a hydroxylic solvent such as methanol.

No decisive arguments for or against the opening of the molecule through a rotation around the C(18)–C(19) bond can be drawn from the comparison, in Table 1, of available experimental ^1H–^1H coupling constants and calculated ones for geometry **IV**. However, Painter's study of carbon relaxation times[2] show that the C(10), C(23) part of the molecule should be rigid. This would mean that an opening of the molecule through motions at the C(18), C(19) level is unlikely. Indeed, enthalpy barriers were found to be higher than for rotation around the C(8)–C(9) bond. Geometries of types **IV** or **V** would not be favoured in methanol. Geometries of types **II** or **III** in which a strong hydrogen bond system [O(4)H–O(6), O(8)H–O(4), O(8)H–O(7)] is retained in this part of the molecule, would be favoured.

Concerning the lasalocid anion in methanol, it was shown previously[1] that it occurs as a free anion in dilute solutions. The chemical equivalence between protons 8A and 8B is lost and coupling constants in the 8,9 proton system (Table 4) are not far from those recorded in acetonitrile or chloroform. It can thus be expected that the salicylate group is fixed through hydrogen bonding with O(4). From comparison of ^{13}C and ^1H chemical shifts it was suggested[1] that the tail of the molecule can be taken to be in the same conformational state in the acid and anion forms. Hence hydrogen bonding of the carboxylate group to O(8) can also be postulated. The question remains of how this closure is achieved, through O(1)–HO(8), O(2)–HO(8) or both ligations. Comparison of calculated coupling constants for the two closed geometries investigated here, to experimental ones in methanol, is clearly in favour of form I_a'; the fit is in particular better than for I_a, for the tetrahydrofuran ring. Closure through a O(8)H–O(2) hydrogen bond would thus be favoured.

Lasalocid Anion in Water: Monomers and Aggregates.—In water, lasalocid anions in lasalocid tetraalkylammonium salts were shown[10] to occur as free monomers in dilute solutions and as aggregates in concentrated solutions. Critical concentrations are appreciable, of the order of 10^{-2} mol dm^{-3} implying that free anions are well accommodated by water. It can therefore be assumed that their structure is such that all the oxygens of the molecule are readily accessible to water molecules. The computations reported here show that stable structures can be attained on completely unfolding the molecule. The formation enthalpy for geometry VI_a is (Table 4) higher than that of the closed geometries but lower than that of the geometries opened through either C(8)–C(9) or C(18)–C(19) rotations; in this unfolded geometry the steric hindrance between the atoms of the molecule is minimized. The only hydrogen bonds that subsist are O(3)H–O(1) and O(8)H–O(7). The latter one would probably be released in a solvent like water.

It can be observed in Fig. 8 that in the VI_a geometry the oxygens are located on one side of the molecule, and the alkyl group on the other side. This would suggest that aggregates could be formed through interaction of hydrophobic faces of a certain number of lasalocid anions, the oxygens being turned outward, in interaction with water molecules, and some of the tetraalkylammonium cations being fixed in such charged cylinders. This picture would be consistent with our finding[10] that through aggregation ^{13}C resonances of the alkyl groups of the lasalocid anion shift downfield whereas that of carbons and hydrogens neighbouring the carboxylate group shift upfield.

This postulated aggregate structure is clearly only one of the possible ones. Strong solvation of the monomers by water probably does not require total unfolding of the anion molecule, and other structural types of aggregation may occur. Structures of both monomers and aggregates are being investigated using various experimental methods.

Conclusions

Geometries of lasalocid acid and anion corresponding to expected conformations in the solid state and in various solvents were generated using various procedures. The high motility of the lasalocid structure is confirmed. Even for the closed form a choice can be made between various computed conformations, which was suspected from appreciably solvent-variable $^1H-^1H$ coupling constants. Clearly, the possible conformations are more numerous than those selected here. It is, however, likely that the geometries obtained here are representative of lasalocid at least in the states and solvent systems investigated to date.

This study underlines the limits to the use of coupling constant to relate computed and experimental conformations: owing to overcrowding some of the relevant constants could not be reached; dihedral angles for bonds involving quaternary carbons cannot be estimated; in addition, the choice of the formula used is always questionable. Concerning lasalocid acid and anion we are expecting to determine distances from NOE. $^1H-^1H$ experiments in various situations which would allow more precise fitting.

One of the main outcomes of this work is that it provides starting structures for studying the complexation of cations by both ligands, lasalocid acid and anion, in various situations. Such complexes, as well as these two ligands, could be investigated by various computational methods (*e.g.* quantum semi-empirical, statistical mechanics and molecular dynamics) both in the vacuum state and in solution. Our purpose would be to link the results so obtained to the spectroscopic and thermodynamic features observed, mainly those reported previously in this series of papers.

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