Interactions between Metal Cations and the Ionophore Lasalocid. Part 9.[1] Structural Study of the Free Acid, Anion and Potassium Complex Salt in Chloroform and Methanol, using ^{13}C and ^{1}H Nuclear Magnetic Resonance Spectroscopy†

Rachid Lyazghi,[a] Annie Cuer,[b] Gérard Dauphin[b] and Jean Juillard*[,a]
[a] *Laboratoire de Chimie Physique des Solutions, UA CNRS 434, Université Blaise Pascal (Clermont), 63177 Aubière Cédex, France*
[b] *Laboratoire de Chimie Organique Biologique, UA CNRS 485, Université Blaise Pascal (Clermont), 63177 Aubière Cédex, France*

From two-dimensional NMR experiments, complete and unequivocal assignment of the proton and carbon resonance shifts is independently obtained for the free acid, anion, and potassium salt of lasalocid in both chloroform and methanol. Variation of the ^{1}H and ^{13}C chemical shifts of the anion and the potassium salt in chloroform as a function of their concentration afforded both their dimerization constants and the NMR parameters specific to both monomers and dimers. From the present results, mainly ^{1}H and ^{13}C chemical shifts and ^{1}H–^{1}H coupling constants, and other data involving various methods, the state and structure of the various species formed in the two solvents (conformation of lasalocid, co-ordination sites of the cation, solvation, *etc.*) are discussed. The high flexibility and adaptability of lasalocid is stressed.

Lasalocid is a polyether polycyclic carboxylic ionophore of bacterial origin. Its structure, showing the carbon and oxygen numbering-scheme is shown in Fig. 1. Owing to its ability to help monitoring of ion transportation across membranes it has been widely used in the study of some fundamental biological processes. It also has some industrial importance as a ration additive in cattle feed and as a drug in the treatment of poultry coccidiosis. As with the other ionophores of this family, progress in the understanding of its mechanism of action depends on a better knowledge of the thermodynamics and kinetics of the complexation–decomplexation process in systems simpler than living ones. We are engaged on a systematic study of the thermodynamic parameters related to the complexation of a wide variety of metal cations by the ionophore lasalocid in both homogeneous media (parts 1–8 of this series) and heterogeneous systems (parts 10–12). Two reaction media are currently under investigations: the solvent methanol which readily solubilizes the metal salts, the ionophore lasalocid, and its complexes, and the water–chloroform system in which the metal salt resides in the aq. phase, and the ionophore and its complexes in the organic phase.

Both complexation and transport of the cations are greatly influenced by the structure of the ligand and the complex species formed. Hence a systematic study of the various lasalocid anion–metal cation complexes in both methanol and chloroform, by using ^{13}C and ^{1}H NMR spectroscopy, was undertaken. The information expected to be obtained included the stoicheiometry of the complexes formed, the bonding sites involved in the complexation, and the conformation of the ligand moiety in the complexes, *i.e.* the lasalocid anion. The present report focusses on simple species, free lasalocid acid, its anion form, and the lasalocid potassium salt in both $CDCl_3$ and CD_3OD.

As regards the lasalocid free acid form, partial assignment of the proton and carbon resonance shifts has been reported by Patel and Shen for both chloroform[2] and methanol.[3] The proton spectra in an aprotic solvent were mainly elucidated by Anteunis[4] using homo-INDOR and double-irradiation measurements. Complete assignment of the ^{13}C NMR spectra in CD_2Cl_2 was achieved by Seto *et al.*[5] using model compounds and biosynthetically enriched samples of lasalocid. A slightly modified set of ^{13}C chemical shifts was later proposed by Lallemand and Michon.[6] However, ambiguities are not always resolved and such assignments using only one-dimensional methods have, in some cases, to be used with some caution. All these data allowed comparison of conformations in solution with the known structures of lasalocid acid or its bromo derivative in the solid state.[7,8] ^{13}C Relaxation experiments have suggested that the free acid is a monomer in the two solvents[2,3,9] and that marked conformational changes occur when solvent polarity varies from chloroform to methanol.[9]

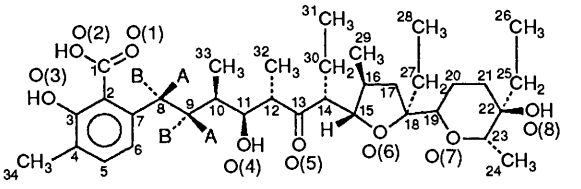

Fig. 1 Lasalocid structure showing the carbon and oxygen numbering scheme

No ^{1}H and ^{13}C NMR data concerning the lasalocid potassium salt have yet been reported. However, much information is available on the sodium salt since its first study in the solid state and in solution by Schmidt *et al.*[10] Assignment of ^{13}C[5,6,10] and ^{1}H[4,10] resonance shifts in chloroform were proposed. Data are scarcer in methanol; only partial assignment of the ^{13}C[3,9] and ^{1}H[3] chemical shifts have been reported for the sodium salt. From ^{13}C relaxation studies[3,10] this salt was presumed to be dimeric in aprotic media but monomeric in methanol. Solids crystallized from various solvents were shown to be dimeric[10,11] but a monomeric form was crystallized from methanol.[8] Information concerning the silver salt both in solution[2] and in the solid salt[12] is irrelevant here, given the

† In partial fulfilment of the *Thèse d'Université* of R. Lyazghi, Université Blaise Pascal, 1991.

specific interaction between the silver(I) ion and the aromatic ring. However, a very interesting study concerning the thallium(I) salt in chloroform was made by Lallemand and Michon.[6] A coupling process was observed at low temperature between the thallium nucleus and some of the carbon nuclei; this enabled the oxygen atoms involved in the bonding of the thallium ion to be fairly confidently assigned.

Little is known about the lasalocid free anion form in solution; only some proton and carbon chemical shifts and relaxation times in methanol have been reported by Shen and Patel.[3] Therefore the solution state of this species is not as well documented as others, which is surprising since most of the lasalocid–metal complexes studied involved the lasalocid anion as ligand.

The present research was, therefore, undertaken with the aim of obtaining more insight into the conformation of the free lasalocid acid in solution and of assessing complete NMR parameters and thereby the conformations of the free anion in methanol and the ion-pair engaged anion in chloroform. For alkali-metal complexes, the potassium salt was preferred in initial studies, owing to the better selectivity of lasalocid for potassium than for sodium in all the solvent media investigated[13] and the lack of NMR data for this particular species.

Our purpose was to achieve complete assignments of the ^{13}C and ^{1}H resonance shifts using two-dimensional NMR methods, ^{1}H–^{1}H homocorrelations (COSY), ^{13}C–^{1}H short-range and, if necessary, long-range hetero-correlations, together with ^{1}H and ^{13}C broad-band as well as *J*-modulated spin echo one-dimensional spectra. Most of the ^{1}H–^{1}H apparent coupling constants could then be obtained with some reliability from the various spectra, and the conformations then assessed. In addition, ^{13}C relaxation experiments were conducted for the anion form in order to document the flexibility of this species in solution. NOE effects were not studied since they cannot be assessed through NOESY experiments (ROESY experiments are now in progress).

Experimental

Chemicals.—Lasalocid acid was obtained as previously[14] from its commercial sodium salt. The lasalocid tetraethylammonium salt was prepared as described for its tetramethylammonium salt,[15] starting with tetraethylammonium iodide 'puriss' from Fluka A.G. The potassium salt was prepared by exact neutralization of the lasalocid by a solution of potassium methoxide in methanol. After evaporation of the methanol, the salt was dissolved in acetone, and the acetone was then evaporated, this being repeated three times in order to remove last traces of water. After being dried in a vacuum oven, the purity of the potassium salt was checked by titration in methanol against a standardized perchloric acid solution and by atomic spectroscopy. The potassium methoxide solution was obtained and standardized as previously.[16]

The methanol used in the conductivity experiments was prepared as previously.[14] The chloroform used for the UV experiments was a product *'pro analysi'* from Merck A.G., Darmstad, Germany. [$^{2}H_4$] Methanol and deuteriochloroform used in NMR experiments were of the best quality grade and were obtained from the CEA (France).

NMR Spectra.—All the spectra were recorded on a Bruker *MSL 300* instrument.

COSY. The applied pulse sequence was $(\pi/2)$–(t_1)–$(\pi/4)$–(FID, t_2). The spectral width in F_1 and F_2 was chosen in order to detect all the protons except the aromatic ones; the number of data points in t_2 was 2048 and from 118 to 512 increments were recorded. Before Fourier transformation, zero scaling was applied in t_1 (size S_{i1} = 1024) and the data were multiplied by the unshifted sine-bell function. Total acquisition times ranged from 4 to 10 h. The $\pi/2$ pulse time was 7 μs.

^{1}H–^{13}C *Shift correlation.* The applied pulse sequence was $(\pi/2,\ ^{1}H)$–$(t_1/2)$–$(\pi,\ ^{13}C)$–$(t_1/2)$–(τ_1)–$(\pi/2,\ ^{1}H;\ \pi/2,\ ^{13}C)$–$(\tau_2)$–(BB, ^{1}H; FID, t_2) with τ_1 = 0.003 57 s and τ_2 = 0.001 785 s. The spectral width in F_1 was as for the COSY spectra, and that chosen for F_2 was in order to detect all the carbons except the aromatic ones, and C-1 and C-13; the number of data points in t_2 was 2048 and 128 S_{i1}, 256 or 512 increments were recorded. Before Fourier transformation, zero scaling was applied in t_1 (size S_{i1} = 1024) and the data were multiplied (unshifted sine-bell) in F_2 and exponential in F_1. The total acquisition time ranged from 10 to 24 h. The $\pi/2$ pulse was 3.5 μs for ^{13}C and the decoupler $\pi/2$ pulse was 10 μs. The same procedure was used for ^{1}H–^{13}C long-range shift correlations except that τ_1 and τ_2 were adjusted in order to determine a maximum polarization for J_{C-H} = 8 Hz; τ_1 = 0.06 s and τ_2 = 0.03 s.

T_1 *Determinations.* These were obtained by the inversion–recovery method using the usual sequence (π)–t_1–$(\pi/2)$–t_2 with variable t_1.

Results

Resonance Chemical Shift Assignments.—Assignments of lasalocid ^{13}C and ^{1}H chemical shifts were made independently for each species in each solvent. The proton spectrum and the broad-band carbon spectrum, along with the *J*-modulated spin-echo spectrum, which allowed the various carbon atoms of the molecule to be classified according to their parity, were first recorded. Assignments in the downfield region were easily made directly from these spectra according to the usual principles and in agreement with the findings of previous experimenters. For ^{1}H chemical shifts lower than δ_H = 4.5 and ^{13}C chemical shifts lower than δ_C = 100 more thorough investigations were conducted. 1-D NMR methods cannot afford a total assignment. As one of us demonstrated elsewhere,[17] 2-D NMR spectra can solve this problem. ^{1}H–^{1}H Chemical-shift correlation (COSY) provided a first and somewhat partial assignment of the proton, and then ^{1}H–^{13}C chemical-shift correlations gave a first assignment of the carbon resonances and backed up the ^{1}H assignments. These procedures enabled us, in each case, almost completely to unravel the ^{1}H and ^{13}C spectra. The only ambiguity which generally subsisted concerned the assignment of methylenes 25 and 27 and of methyls 26 and 28. This was resolved by using long-range ^{1}H–^{13}C spectroscopy. Results thus obtained are reported in Tables 1 and 2.

Most of the previous data concerned the lasalocid free acid form in aprotic solvent. Our results agree very closely with ^{13}C assignments by Seto *et al.*[5] but discrepancies occur with Painter's data[9] (C-20, C-21 inversions) and results obtained by Lallemand and Michon[6] (numerous inversions in the methyl and even in the methylene region). A complete set of ^{1}H assignments had been reported before only by Anteunis.[4] The agreement between his values and ours is fair except in the more crowded regions of the ^{1}H spectrum in which numerous inversions are observed.

^{1}H–^{1}H *Apparent Coupling Constants.*—These were obtained using various methods. Concurrently, the assignments of the ^{1}H chemical shifts were confirmed and sometimes completed. Four different methods were used: (i) determination of the coupling constants from the simple one-dimensional spectra for well isolated protons and well defined coupling; (ii) recording of one-dimensional spectra under irradiation of one or more protons; this was systematically done in any doubtful case; (iii) assignment and unravelling of overlapping multiplets

Table 1 Experimental ^1H chemical shifts (δ) for the free acid, the anion, and the potassium salt of the ionophore lasalocid in $CDCl_3$ and CD_3OD at room temperature for concentrations of ~0.2 mol dm^{-3}, accuracy ±0.01 ppm

	Tetraethylammonium salt		Free acid		Potassium salt	
Proton	$CHCl_3$	MeOH	$CHCl_3$	MeOH	$CHCl_3$	MeOH
5	6.87	7.03	7.18	7.24	6.98	7.05
6	6.37	6.57	6.65	6.74	6.40	6.60
8A	3.70	3.36	3.36	3.05	3.92	3.97
8B	2.19	2.94	2.49		2.05	2.38
9A	1.63	1.73	1.70	1.70	1.61	1.67
9B	1.26	1.55	1.46		1.47	1.50
10	1.57	1.75	1.70	1.73	1.60	1.70
11	4.04	3.99	4.14	3.92	4.31	4.40
12	2.79	3.04	2.82	3.05	2.78	2.94
14	2.50	2.83	2.60	2.84	2.47	2.78
15	3.80	3.87	3.90	3.88	3.87	3.95
16	1.96	2.14	2.19	2.17	2.16	2.23
17A	1.77	1.88	1.88	1.89	1.80	1.97
17B	1.34	1.71	1.43	1.66	1.32	1.48
19	3.36	3.60	3.48	3.59	3.40	3.62
20A	1.77	1.73	1.90	1.74	1.62	1.94
20B	1.34	1.53	1.50	1.53	1.41	1.54
21A	1.63	1.64	1.68	1.70	1.76	1.75
21B	1.47	1.50		1.53	1.56	1.52
23	3.66	3.82	3.95	3.83	3.86	3.68
24	1.09	1.22	1.20	1.25	1.05	1.26
25A	1.34	1.38	1.63	1.39	1.36	1.57
25B	1.17		1.37			1.40
26	0.85	0.97	0.97	0.92	0.97	1.02
27A	1.54	1.67	1.70	1.64	1.52	1.88
27B	1.26		1.40		1.09	
28	0.82	0.91	0.83	0.90	0.65	0.95
29	0.93	1.06	1.07	1.08	1.01	1.15
30A	1.82	1.97	1.92	1.91	1.85	2.10
30B	1.39	1.57	1.35	1.56	1.18	1.46
31	0.77	0.93	0.85	0.91	0.76	0.88
32	0.87	0.99	0.95	1.02	0.93	1.06
33	0.72	0.94	0.91	1.01	0.78	0.92
34	2.07	2.19	2.23	2.26	2.20	2.14

corresponding to sets of protons with neighbouring chemical shifts from the shape of the spots in the ^1H–^1H correlation (COSY) spectrum; (iv) use of ^{13}C–^1H correlation lines drawn for each carbon which provided an isolated spectrum for each proton.

By using and combining these four methods we obtained the apparent coupling constants for most of the geminal and vicinal proton coupling; only a few of these could not be assessed. Results obtained independently for the three species in the two solvents are given in Table 3. Our results for the potassium salt (AK) in chloroform compare favourably, with some small expected differences, with Anteunis' data for the sodium salt (ANa) in chloroform, once this author's few erroneous chemical-shift assignments are corrected.

Monomer–Dimer Equilibria.—X-Ray diffraction studies of solid-state crystals of lasalocid[7] and its monovalent cation salts[10–12] obtained from various media have shown that, in most cases, these species are dimeric. One of the questions raised in the earlier NMR studies in solution[2,3,10] was whether lasalocid and its salts occur as monomers or dimers in aprotic as well as in protic solvents. From a ^{13}C relaxation study, Painter[9] concluded that, in chloroform, lasalocid free acid is monomeric and that its carbon backbone is rigid and can be characterized by a single rotational correlation time. We have recorded ^1H spectra for lasalocid in chloroform for concentrations ranging from 10^{-1} to 10^{-4} mol dm^{-3}. No appreciable changes (>0.03 ppm) were observed for the easily assigned chemical shifts. This would confirm the monomeric state of the acid form of lasalocid at least for concentrations up to 10^{-1} mol dm^{-3} in chloroform.

In methanol the existence of dimers is less likely given the magnitude of the rotational correlation time[9] and also all the results obtained using potentiometry; see, for example, ref. 14. In addition, crystals of monomeric lasalocid species have been isolated from methanol solution.[8]

It has been suggested, first by Shen and Patel,[3] from ^{13}C relaxation data, that the sodium salt exists as a monomer in methanol but as a dimer in chloroform. The potassium salt was then suspected of behaving similarly and the variation (with concentration) of the ^{13}C and ^1H chemical shifts of the potassium salt in chloroform was expected to provide some information on the monomer–dimer equilibria. A systematic investigation was therefore conducted for concentrations ranging from 5×10^{-1} to 5×10^{-3} mol dm^{-3}. Stronger variations were obtained for 28-H, which varied from δ_H 0.63 to δ_H 0.74; further investigations for concentrations of 10^{-4} to 5×10^{-5} mol dm^{-3} give, respectively, δ_H 0.81 and 0.82, values which should be taken with some caution given the high signal-to-noise ratio at these concentrations. The fact that only one well resolved signal was observed suggests that the anionic lasalocid ligands in the two species, monomeric AM and dimeric A_2M_2, are exchanging rapidly. Special attention was devoted to the methyl C-28 of lasalocid sodium salt by Schmidt *et al.*[10] who reported a 0.25 ppm change from chloroform to acetone and noticeable differences, as given here in Table 1, when comparing ^1H chemical shifts of methyls 28 and 31 in chloroform. The same authors have shown that two types of

Table 2 Experimental ^{13}C chemical shifts (δ_C) for various lasalocid species in chloroform and methanol at room temperature for concentrations of ~0.1 mol dm^{-3}, accuracy ±0.1 ppm

	Tetraethylammonium salt		Free acid		Potassium salt	
Carbon	$CHCl_3$	MeOH	$CHCl_3$	MeOH	$CHCl_3$	MeOH
1	173.9	175.9	173.3	175.0	175.9	177.1
2	117.4	119.3	111.2	112.7	116.8	118.1
3	162.4	161.1	161.6	162.1	161.6	161.5
4	122.9	123.4	124.2	124.9	122.9	123.9
5	131.3	132.6	135.0	135.8	131.5	132.9
6	119.0	121.3	121.4	122.5	119.6	121.4
7	144.0	144.8	144.2	145.1	143.8	144.8
8	33.2	34.1	34.3	35.1	32.5	32.8
9	37.8	38.1	36.8	38.1	37.6	37.9
10	34.2	35.6	34.7	35.8	33.5	33.8
11	71.6	75.5	72.6	75.9	70.2	71.8
12	48.0	49.6	48.9	49.9	48.7	49.0
13	215.7	217.8	214.9	217.4	218.3	219.0
14	56.4	57.6	55.4	57.0	56.0	56.6
15	83.4	85.5	83.7	85.6	82.8	84.6
16	36.2	37.8	34.7	37.3	34.2	34.4
17	40.1	41.0	38.9	40.6	37.8	38.1
18	85.3	86.8	86.3	87.1	87.4	88.0
19	72.5	73.5	70.8	73.2	69.1	71.2
20	20.4	22.1	19.7	21.9	19.2	19.5
21	30.5	30.2	30.4	30.3	29.7	30.0
22	70.6	72.1	72.6	72.1	70.8	72.2
23	76.4	77.8	76.1	77.8	76.6	77.5
24	14.1	14.8	13.8	14.7	13.4	13.6
25	31.4	32.2	29.9	32.1	31.7	32.0
26	6.6	6.8	6.4	6.7	6.5	6.8
27	29.5	30.8	30.6	30.8	29.8	29.9
28	8.8	8.8	9.0	8.8	9.2	9.5
29	16.2	16.9	15.6	16.5	15.8	15.8
30	17.5	19.7	16.6	19.1	16.1	15.9
31	12.8	12.7	12.8	12.8	12.0	12.3
32	13.5	14.2	13.0	14.1	13.2	12.8
33	13.3	13.0	13.2	12.6	12.6	13.4
34	16.2	16.2	15.8	15.9	16.2	16.4

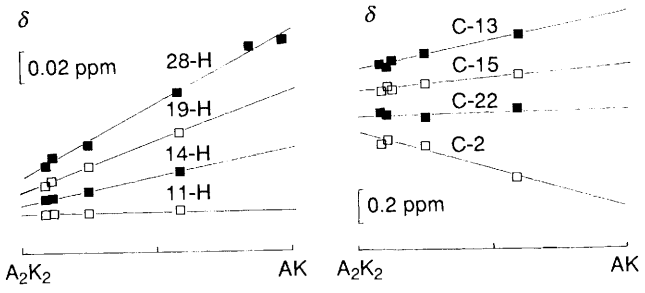

Fig. 2 Chemical shifts, in chloroform, of various protons and carbons of lasalocid potassium salt as a function of the monomer ratio according to relation (4). ■, □ experimental points; —— line expected using K_D = 125 and the parameters in Tables 4 and 5; data vertically shifted for clarity.

structure for the dimer of the sodium salt occur in the solid state, corresponding to two crystal modifications: a 'head-to-tail' structure and a 'head-to-head' structure. In the 'head-to-tail' structure the methyl C-28 of one lasalocid anion is located in front of the benzene ring of the other lasalocid anion. If such a structure occurs in chloroform solution, the abnormal upfield shift observed might be caused by anisotropy due to an aromatic ring current.

Processing the variation of the 28-H chemical shift with concentration provided the dimerization constant K_D [eqn. (1)] corresponding to reaction (D) where c_1 and c_2 stand,

$$2\,AK \rightleftarrows A_2K_2 \qquad (D)$$

$$K_D = c_2/c_1^2 \qquad (1)$$

respectively, for actual concentrations of the monomer and dimer.

The experimental chemical shift δ is related to the 28-H chemical shifts in the monomer δ_1 and in the dimer δ_2 as given by eqn. (2), where c^* is the total ionophore concentration,

$$c^*\delta = c_1\delta_1 + 2\,c_2\delta_2 \qquad (2)$$

given by eqn. (3), which yields eqn. (4).

$$c^* = c_1 + 2\,c_2 \qquad (3)$$

$$\delta = (\delta_1 - \delta_2)c_1/c^* + \delta_2 \qquad (4)$$

Solving this system for the 28-H proton gave best results for K_D = 125 (molar scale of concentration; room temperature; estimated accuracy ± 25), δ_1 = 0.822 and δ_2 = 0.620 (estimated accuracy ± 0.010 ppm). Although weaker, the variation (with dilution) of some other ^{13}C and ^1H nuclei of the molecule is, through the dimerization process, significant. By using eqn. (2) and values of K_D obtained here it was possible to determine, from our spectra at various concentrations, the chemical shifts corresponding to the monomeric and dimeric forms of the lasalocid potassium salt in chloroform. Typical examples of such a variation of the ^1H and ^{13}C chemical shifts with the concentration of the monomer are given in Fig. 2. δ_1 and δ_2 for some selected protons, those easily assessed through examination of the one-dimensional ^1H spectra, are reported in Table 4. Analogous data for all the carbons of the ionophore are gathered in the two first columns of Table 5. By

Table 3 Apparent coupling constants ^3J and ^2J (Hz) between neighbouring protons of lasalocid for the acid, the anion, and the potassium salt in chloroform and methanol at room temperature for concentrations of ~0.1 mol dm^{-3}, accuracy ±0.1 Hz

	$CHCl_3$			MeOH		
J_{H-H}	AH	$ANEt_4$	AK	AH	$ANEt_4$	AK
5–6	7.5	7.5	7.2	7.4	7.5	7.7
8A–8B	12.8	12.5	12.0		11.2	11.4
8A–9A	3.2	2.5	2.0		4.0	2.8
8A–9B	12.8	12.5	12.0		11.2	11.4
8B–9A	12.8	12.5	12.0		11.2	11.4
8B–9B	5.0	5.6	4.5		5.6	6.0
9A–9B	12.8	12.5	12.0		~11	11.4
10–9A		~4.0			Σ7.5	≦2.5
10–9B		~2.0	~5			~4
10–33	7.0	7.0	7.0	6.3	6.5	6.5
11–10	1.6	1.2	1.2	1.5	1.8	1.8
11–12	9.6	10.0	9.8	9.5	10.0	10.2
12–32	7.0	7.0	7.0	7.0	7.0	7.0
14–30A	10.5	10.0	10.8	9.6	10.0	10.5
14–30B	2.5	2.5	2.1	3.2	4.0	2.5
14–15	2.4	2.5	2.0	3.2	4.0	2.5
15–16	10.4	10.4	10.0	10.0	10.0	10.2
16–17A	7.7	5.0	6.0	7.5	8.0	7.7
16–17B	12.6	10.5	11.0	11.5	11.0	11.2
16–29	6.5	6.5	7.0	6.5	6.5	6.5
17A–17B	12.6	12.6	13.0	12.7	12.6	12.6
19–20A	12.0	11.2	12.0	10.5	10.0	12.0
19–20B	2.4	1.6	1.5	3.0	3.2	2.1
20A–20B	14.7	12.6	13.0	13.0	14.0	11.2
20A–21A	4.3	9.0	11.0		~11	11.2
20A–21B		~3.0	6.0	5.0	~5	4.6
20B–21A	<	~4.5	6.0	6.5	~6	5.5
20B–21B	<	<	2.5			
21A–21B		12.6	13.0	11.2	~12	11.4
23–24	7.0	7.0	7.0	7.0	7.0	7.0
25(A,B)–26	7.0	7.0	7.0	7.0	7.0	7.0
25A–25B	14.0	14.0				14.0
27(A,B)–28	7.5	7.0	7.2	6.7	6.8	7.0
27A–27B		14.0	14.0			
30(A,B)–31	7.5	7.0	7.0	7.0	6.8	7.0
30A–30B	14.0	12.0	14.2	14.0	14.0	13.5

using the reported value of the dimerization constant it was calculated that the proportion of the monomer was ~14% in the main experiment conducted with a roughly 0.1 mol dm^{-3} solution. ^{1}H and ^{13}C Chemical shifts of the lasalocid potassium salt reported in Tables 1 and 2 are not, therefore, very different from those given in Table 4 and 5 for the dimer.

The tetraethylammonium salt of lasalocid was assumed to be strongly dissociated in methanol. This was consistent with our previous results[14] concerning the potentiometric titration of the ionophore acid against methanolic tetraethylammonium methoxide and was confirmed by conductivity. Both types of measurements showed that this salt is a strong electrolyte in methanol. Conductivity measurements also showed that there is no dimerization of the free anion giving rise to species such as A_2^{2-}, most unlikely anyway in a protic solvent.

Things were not so clear for the lasalocid anion in chloroform. This prompted us to study the variation of the proton and carbon chemical shifts with the concentration of lasalocid tetraethylammonium salt. The most marked variations were found to occur for proton 5-H. A good fit to eqn. (4) was obtained using $K_D = 20$ (molar scale of concentration; room temperature; estimated accuracy ± 10), $\delta_1 = 7.10$ and $\delta_2 = 6.79$ (estimated accuracy ± 0.02 ppm). Use of this K_D-value and the spectra at various concentrations then afforded, as previously for the potassium salt, the chemical shifts for the monomer and the dimer anion, δ_1 and δ_2, of all the carbons and some of the hydrogens. These are reported in the last columns of Tables 4 and 5. From these results the data reported in Tables 1 and 2 would correspond to a 30% monomer–70% dimer mixture. As regards the coupling constants, given the accuracy of their determination and the weakness of the variation observed between the various species, no attempt was made to assess proper values for monomers and dimers. Therefore, values reported in Table 3 for the potassium and tetraethylammonium salts of lasalocid must be considered as

Table 4 Some ^{1}H chemical shifts for the monomer, δ_1, and the dimer, δ_2, of the anion and of the potassium salt of lasalocid in chloroform (room temperature)

Proton	AK δ_1	A_2K_2 δ_2	A^- δ_1	A_2^{2-} δ_2
5	7.06	6.94	7.10	6.79
6	6.52	6.39	6.56	6.27
8A	4.00	3.85	3.87	3.60
11	4.32	4.32	4.16	3.99
12	2.80	2.75	2.82	2.82
14	2.54	2.46	2.54	2.38
15	3.92	3.89	4.04	3.72
19	3.53	3.34	3.54	3.25
23	3.87	3.87	3.84	3.58
24	1.18	1.03	1.16	1.07
26	0.96	0.96	0.94	0.79
28	0.83	0.61	0.87	0.69
29	1.06	0.99	1.06	0.88
31	0.82	0.74	0.87	0.73
32	0.92	0.92	0.97	0.77
33	0.80	0.78		

Table 5 ^{13}C Chemical shifts for the monomer, δ_1, and the dimer, δ_2, of the potassium salt of the anion in chloroform (room temperature)

Carbon	AK δ_1	A_2K_2 δ_2	A^- δ_1	A_2^{2-} δ_2
1	176.0	175.9	174.1	173.7
2	115.8	116.9	117.1	117.3
3	162.2	161.5	162.5	162.1
4	123.8	122.9	123.4	122.6
5	132.5	131.4	131.9	131.1
6	120.0	119.6	119.4	118.9
7	144.1	143.6	144.2	143.9
8	33.2	32.4	33.5	33.0
9	37.9	37.6	37.7	37.6
10	33.8	33.5	34.4	34.0
11	70.7	70.2	71.5	71.5
12	49.3	48.7	48.4	47.8
13	219.1	218.2	215.8	215.6
14	56.4	56.0	56.3	56.1
15	83.2	82.7	83.1	83.5
16	34.9	34.1	35.8	36.2
17	37.8	37.8	39.9	39.9
18	87.5	87.1	85.7	85.2
19	69.6	69.2	72.4	72.1
20	19.9	19.2	20.2	20.4
21	30.1	29.6	30.9	30.2
22	70.9	70.8	70.9	70.4
23	76.7	76.7	76.4	76.4
24	13.8	13.4	14.3	14.0
25	31.9	31.7	31.8	31.1
26	7.0	6.5	6.8	6.5
27	30.0	29.7	29.9	29.3
28	9.6	9.2	9.2	8.7
29	16.2	15.5	16.4	16.1
30	16.3	16.2	17.0	17.7
31	12.3	12.0	13.1	12.5
32	13.5	13.2	13.4	13.4
33	12.7	12.6	13.4	13.1
34	15.9	15.6	16.1	16.1

representative mainly of the dimers. The longitudinal relaxation times T_1 of the carbons of lasalocid tetraethylammonium salt were also determined by using the method of inversion–recovery. NT_1 Values of where N stands for the number of hydrogens bound to the carbon concerned, are, for the carbons of the main backbone of the lasalocid, *i.e.* from C-5 to C-23, remarkably constant: 0.29–0.30 s. In the case of the free acid in chloroform, Painter has shown that T_1 was dominated by a dipolar contribution, and reported NT_1-values for the same carbons in the order of 0.55–0.58 s. Such a difference between acid and anion in the same solvent (chloroform) can also be taken as an indication of the dimerization of the lasalocid anion, although some of the conditions necessary[9] to enable us to relate NT_1 to the effective correlation time of rotation of the molecule cannot be proved to have been fulfilled here, from the present data.

Whatever it may be, lasalocid tetraethylammonium salt appears not to occur in chloroform as a complex or as free ions but as ion multiplets in which anions and cations are associated through electrostatic interactions: the UV spectrum is clearly representative of the anion form; there is a shift of the *C*-band[18] from 309 nm in the potassium salt to 307 nm in the tetraethylammonium salt. Moreover, the high relaxation times of the methylene (NT_1 1.34 s) and of the methyl (NT_1 4.90 s) carbons of the tetraethylammonium ion would suggest an independent rotational motion of this cation with regard to the lasalocid anion. Beside ion-pairs [A^- NEt_4^+], ions-triplets [A_2^{2-}, 2 NEt_4^+] are then believed to be formed. Chemical shifts of both carbons and protons of the tetraethylammonium ion also vary with the concentration of the lasalocid tetraethylammonium salt. Going from monomer to dimer would correspond to the following changes; from δ_C 8.9 to 7.0 for CH_3, from δ_C 54.3 to 51.7 for CH_2, from δ_H 2.1 to 0.9 for CH_3, from δ_H 3.7 to 3.0 for CH_2. Restrictions in the degrees of freedom of the cation would then not be the same through association with monomeric or dimeric lasalocid anion.

Discussion

Lasalocid Free Acid.—It is therefore established that lasalocid free acid is monomeric in both solvents, methanol and chloroform. The lasalocid molecular structure is built from a salicylic sub-unit onto which a long hydrocarbon chain is substituted. The only possible rotations along this chain are located around the bonds C-7–C-8, C-8–C-9, C-9–C-10, C-10–C-11 and C-14–C-15. We shall discuss successively all these possible rotors in the light of data on ^{13}C and ^1H chemical shifts (Tables 1 and 2) and ^1H–^1H coupling constants (Table 3).

In methanol, the chemical equivalence between protons 8_A and 8_B suggests fast interconversion between rotamers. Such an increased rotational freedom around the C-8–C-9 bond, which was suggested by Painter[9] from the significant differences in the ^{13}C relaxation time in the salicylic moiety and in the rest of the molecule backbone, is thereby here confirmed. This is not true for lasalocid in chloroform; rotation is hindered here as shown by the existence of distinct signals in the NMR spectrum, for protons 8_A and 8_B. We suggest from the present work that C-7–C-8 and C-9–C-10 bonds are antiperiplanar and that, as previously reported by Anteunis, the aromatic-ring plane bisects the angle formed by the C-8–C-9 and C-8–8-H_B bonds, this angle being less than 60°. The conformation of the chain from C-7 to C-10 is thus largely different in the two solvents.

Differences are smoother from C-10 to C-15. Values of the J(10-H–11-H) coupling constants (1.8 Hz in methanol and 1.2 Hz in chloroform) suggest that in both solvents the torsion angle between C-10–10-H and C-11–11-H is nearly 90°. On the other hand, coupling constants between protons 11-H and 12-H reflect an antiplanar situation for these two protons. Low coupling between 14-H and 15-H in both both solvents puts these two protons at an angle between 60 and 90 ° to each other. The main differences in this area concern the ^{13}C chemical shift of C-11 and C-13, lower by 3 ppm units in chloroform than in methanol, which reflects the strong solvation of both O-4 and O-5 oxygens by methanol, to which could correspond internal hydrogen bonding between O-4 and O-5 in chloroform.

A tetrahydrofuran (THF) cycle conformation can be inferred from the coupling constants of proton 16-H with protons 15-H, 17-H_A, 17-H_B and 29-H. From these results we suggest that in the two solvents this ring is in an envelope form, its apex being C-16, the protons 16-H and 17-H_A being *cis* (pseudoaxial) and the proton 17-H_B *trans* (pseudoequatorial), which differs somewhat from Anteunis' suggestion of a twist conformation with C-16 above and C-17 below the mean plane. This is true for the two solvents but appreciable differences appear in all the ^{13}C chemical shifts as well as in some of the ^1H–^1H coupling constants J(16-H–17-H_B). Such differences could be related to both a strong interaction of methanol with O-6 and small conformational changes of the ring.

Values obtained for coupling constants J(19-H–20-H_A) $\geqq$ 10.5 Hz and J(19-H–20-H_B) $\leqq$ 3 Hz imply a chair conformation for the tetrahydropyran (THP) ring, protons 19-H and 20-H_A being axial, 20-H_B equatorial. Owing to steric hindrance it is more than probable that methyl 24 and hydroxyl O(8)–H are axial. Differences in ^1H and ^{13}C chemical shifts in the two solvents are small except for C-23 which shifts downfield from chloroform to methanol, this being related to a strong interaction between O-7 and the methanol protons.

If we consider lateral ethyl groups, chemical shifts for protons 30_A and 30_B are clearly distinct in the two solvents; protons 25_A and 25_B as well as 27_A and 27_B apparently have the same chemical shift in methanol but not in chloroform. This could be related to some differences in the degrees of freedom of these two groups in the two solvents.

Therefore, from this and previous data it appears that lasalocid, in chloroform solution, is in a globular form; hydrogen bonding between O(8)–H and one of the carboxyl oxygens ensures the closure of the pseudo-ring and so a relatively constricted structure. This structure was described in detail by Anteunis;[4] our findings largely agree with this description, except for the conformation of the THF ring. The structure in methanol appears to be different. The pseudo-ring is open, which results in a large freedom for the salicylic arm, *i.e.* the molecule backbone from C-1 to C-10 and a looser conformation from C-11 to C-23, which corresponds to more freedom of the final ethyl group but also of the one branched between the two heterocycles. Other differences concern the orientation of all the oxygens in order to facilitate their solvation by methanol, which results in small but definite conformational changes in this area.

Comparison with solid-state data must be done cautiously. Most of the X-ray studies concern dimeric forms of lasalocid or parent compounds. Only one sort of monomeric crystal has been isolated: a 1:1 complex with methanol obtained from a methanolic solution of the ionophore.[8] Both X-ray[8] and IR[19] spectroscopic studies have shown that the pseudo-ring in this complex is closed by an O(2)–H–O(8) hydrogen bond and that the methanol molecule is fixed through hydrogen-bonding with O-1, O-6 and O-8 and so helps determine the conformation of lasalocid. The situation in chloroform is not comparable here since there is no methanol molecule nor even a water molecule in our lasalocid samples; thus other hydrogen bonds could occur: in particular, ligation between O-4 and O-5. Another question is how the pseudo-ring is closed, through O(8)–H–O(2)

or O(8)–H–O(1) buttoning, O-1 being the carboxylic oxygen already bound to the phenolic oxygen O-3; no choice could be made from the present NMR data in chloroform but it is suspected that a structure in which oxygens are better protected from the solvent is preferred in aprotic media. We suggest that closure would occur through hydrogen-bonding between O-8 and O-1 contrary to what was observed in solid structures, either monomeric or dimeric, isolated from protic media.

Monomeric Lasalocid Anion.—When passing from lasalocid (AH) to its anion A^- the most marked variations in ^{13}C and ^1H chemical shifts are observed in the salicylic area and correspond to electronic changes related to the ionization of the carboxylic function.

This having been set aside, two striking facts can be noted for lasalocid anion dissolved in methanol. First, no appreciable variations are observed in the tail of the molecule, particularly on the carbons and protons of the tetrahydropyran (THP) ring: also, the apparent isochronism of geminal protons 25A and 25B, and 27A and 27B, is conserved. Thus this part of the molecule can be taken to be in the same conformational state in the acid and anion forms. The second fact is that protons 8A and 8B are no longer equivalent, which corresponds to stronger hindrance of rotation around the C-8–C-9 bond. This could be due either to steric hindrance caused by stronger solvation of the salicylate group or more probably to carboxylate hydrogen-bonding to O-4. This results in small but definite conformational changes in the C-9–C-14 area but also in the THF ring, which are reflected by small variations in these two areas of ^{13}C and ^1H chemical shifts (Tables 1 and 2) and ^1H–^1H coupling constants (Table 3). C-11's Chemical shift is only little affected; in the free acid, O-4 was probably already engaged in hydrogen-bonding, but with a methanolic oxygen.

Going from acid form AH to monomeric anion form A^- in chloroform results in conformational changes all along the chain as shown by the ^{13}C and ^1H chemical shifts. As mentioned before, the structure of the free acid is taken to be closed through head–tail hydrogen-bonding. The main changes from acid to anion could occur from the substitution of a carboxylate for the carboxyl group which strengthens the interactions of oxygens 1 and 2 with the accessible hydroxy protons H–O-3, H–O-8, and perhaps H–O-4. The strengthening of the closure through O(1)–H–O(8) hydrogen-bonding could explain the variations observed for C-19, C-22, and C-25, the most marked chemical shift observed apart from that of the salicylic moiety. This would correspond to both interactions and conformational changes at the level of the last heterocycle. Other weaker changes concern the C-8–C-14 area. There may be a re-organization of the hydrogen-bonding system from O(4)–H–O(5) to O(2)–H–O(4) which determines some torsion–rotation of the C–C bonds in this area, and even small conformational changes of the THF ring.

Monomeric Potassium Salt of Lasalocid.—Capture of potassium in methanol by lasalocid anion resulted in very marked changes in both the ^{13}C and ^1H chemical shifts of the whole molecule. Variations observed for C-1 and C-2 suggest some involvement of the carboxylate in the complexation of the cation. Strong variations were observed in the C-8–C-15 zone, in particular a strong upfield shift of the C-11 resonance, which is probably related to the disruption of the O(4)–H–O(1) hydrogen bond; positive variation of the C-13 chemical shift can be interpreted as being due to participation of O-5 in the complexation of the cation. From both ^{13}C and ^1H chemical shift changes, it is evident that marked conformational changes occur in the THF ring, which would correspond to involvement of O-6. Finally, O-2, -5, and -6, and possibly O-7, seem to participate in ligation of the cation, which would determine a closure of the pseudo-ring through an O(8)–H–O(1) hydrogen bond; loss of apparent isochronism of protons 25-H_A and 25-H_B is consistent with such a closure. The movements which result in both complexation of the cation and head-to-tail closure of the ligand occur mostly in the region C-8 to C-15. No drastic changes, but continuous weak variations of the ^1H–^1H coupling constants, were observed which show that this conformational change affects all the C–C bonds from C-8 to C-12 and C-14–C-15. In addition, both conformational changes of the THF ring and torsion of the C-18–C-19 bond contribute to these processes. Molecular models showed that in the structure proposed here the cation is placed more in a hemisphere than in a sphere of co-ordination, so that contribution of one or two molecules of solvent methanol is more than probable.

In chloroform, as stated above, the monomeric anion is already in a globular form through O(1)–H–O(8) buttoning. The main changes caused in the ^{13}C chemical shift by K^+ capture concern the carboxylate group, carbon C-13, the THF ring, and carbon C-19. We therefore conclude that, as in methanol, the ligation sites of the cation are O-2, -5, -6 and possibly O-7. Participation of O-2 in the co-ordination probably results in a disruption of the O(2)–H–O(4) hydrogen bond. ^1H–^1H Coupling constants were not assessed for the two monomeric species A^- and AK, but from the carbons and the assessible protons chemical shifts it appeared that complexation of K^+ leads to some reorganization through some torsion–rotation of nearly all the C–C bonds of the molecule, particularly in its flexible parts.

The only data on monomeric lasalocid alkali-metal salts in the solid state concern a sodium salt crystallized with a methanol molecule of crystallization.[8] In addition there are some data on the monomeric sub-unit of a chain-polymerized caesium salt.[20] In the first crystal oxygens O-4, -5, -6, -7, and -8 of the ligand as well as the oxygen of the methanol molecule are implicated in the complexation of the sodium ion; hydrogen bonds involving O-1 and O-8, O-2 and -4 and also O-2 and the methanol oxygen contribute to the stability of the structure. In the caesium lasalocid salt crystal[20] co-ordination of the cation is afforded by oxygens O-2, -5, -6, -7, and -8. In addition, the cation is bound to oxygen O-3′ of the following monomeric unit. The cavity is extended from Na^+ to Cs^+, mean O–M^+ distances ranging, respectively, from 2.3 to 2.6 Å and from 2.9 to 3.2 Å. Thus, when the size of the cation increases the subsequent enlargement of the cavity results in substitution of one of the carboxylic oxygens (O-2) for the hydroxy oxygen (O-4) in the co-ordination of the cation, which was observed here in solution for the potassium salt. In addition, O-8 seems to be involved in the co-ordination of both sodium and caesium cations in the solid state. This may also be true in solution for the potassium salt but no evidence for this was found in the experiments described here, either in methanol or in chloroform.

Dimeric Anion and Potassium Salt of Lasalocid.—As stated above a dimeric form, $A_2Na_2{\cdot}2H_2O$, was characterized[11] in a hydrated crystal of lasalocid sodium salt. In addition, two types of dimeric form (head-to-head and head-to-tail) were observed in sodium 5-bromolasalocid crystals.[10] In the first form the stability of the dimer was mainly achieved through co-ordination of the sodium cation of the first monomer unit by the oxygen O-5 of the second monomer unit; in the second form the stability of the dimer was mainly due to ligation of this sodium cation with one of the oxygens of the carboxylate group of the second monomer unit.[10] Compared with solid-state structures a strong highfield shift of proton 28-H of lasalocid sodium salt in solution in low-polar solvents was attributed to formation of a head-to-tail dimer

in which the 28-H proton of one monomeric unit was under the influence of the aromatic ring current of the other monomer unit.

In chloroform, the dimerization of AK results in a marked decrease in the 28-H ^{1}H chemical shift ($\Delta\delta$ 0.22 ppm). Shifts of the other methyl protons were weak ($\Delta\delta < 0.08$ ppm). In addition, perturbations observed for carbons and protons of the salicylate group are rather marked. These two facts suggest a head-to-tail structure for the dimeric potassium salt in which ligation of one cation will be assured by oxygens O-5, -6, -7 and possibly O-2 of one lasalocid anion and the oxygen O-2′ of the other lasalocid anion. Variation of carbon chemical shifts all along the chain, although weak, suggest small conformational rearrangements of the anion ligand in the process of dimerization.

Given the chemical shift obtained for the 28-H proton, lasalocid anion dimer seems also to be a head-to-tail dimer. However, the small variations of the various ^{13}C chemical shifts observed between the two forms, monomer and dimer, do not reliably indicate what the main contact sites are between the two anion molecules in the dimer. Nevertheless, the fact that the anion, and not the acid, dimerizes would suggest involvement of the carboxylate group in this process; a possible ligation process would then be between the carboxylate group of the first anion and the hydroxy group O(4)–H of the second one.

Conclusions.—Lasalocid shows high flexibility. It can adopt markedly different mean conformations depending on the solvent and the ligating species with which it is engaged. Even when a globular form is adopted, the charged groups or atoms are not well protected from the environment, which results, in protic media, in the participation of the solvent in ligation of the charged sites, and in aprotic media in a dimerization which ensures better protection of either the cation or the carboxylate group.

References

1 Part 8, Y. Pointud and J. J. Juillard, *J. Chem. Soc., Faraday Trans.*, 1990, **86**, 3395.
2 D. J. Patel and C. Shen, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 1786.
3 C. Shen and D. J. Patel, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 4277.
4 M. J. O. Anteunis, *Bioorg. Chem.*, 1976, **5**, 327.
5 H. Seto, J. W. Westley and R. G. Pitcher, *J. Antibiot.*, 1978, **31**, 289.
6 J. Y. Lallemand and W. Michon, *J. Chem. Res.*, 1978, (*S*) 162; (*M*) 2081.
7 E. C. Bissel and I. C. Paul, *J. Chem. Soc., Chem. Commun.*, 1972, 967.
8 C. C. Chiang and I. C. Paul, *Science*, 1977, **196**, 1441.
9 G. R. Painter and W. A. Gibbons, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1151.
10 P. G. Schmidt, A. H. J. Wang and I. C. Paul, *J. Am. Chem. Soc.*, 1974, **96**, 6189.
11 G. D. Smith, W. L. Duax and S. Fortier, *J. Am. Chem. Soc.*, 1978, **100**, 6725.
12 C. A. Maier and I. C. Paul, *Chem. Commun.*, 1971, 181.
13 B. G. Cox, Ng Van Truong, J. Rzeszotarska and H. Schneider, *J. Am. Chem. Soc.*, 1984, **106**, 5965.
14 J. Juillard, C. Tissier and G. Jeminet, J. Chem. Soc., *Faraday Trans. 1*, 1988, **84**, 951.
15 J. Woźnicka, C. Lhermet, N. Morel-Desrosiers, J.-P. Morel and J. Juillard, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 1709.
16 Y. Pointud, C. Pierzo, C. Tissier and J. Juillard, *J. Chim. Phys.*, 1985, **82**, 891.
17 J. C. Beloeil, M. A. Delsuc, J. Y. Lallemand, G. Dauphin and G. Jeminet, *J. Org. Chem.*, 1984, **49**, 1797.
18 Y. Pointud, E. Passelaigue and J. Juillard, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 1713.
19 J. M. Friedman, D. L. Rousseau, C. Shen, C. C. Chiang, E. N. Duesler and L. C. Paul, *J. Chem. Soc., Perkin Trans. 2*, 1979, **3**, 835.
20 W. F. Paton and I. C. Paul, cited by E. N. Duesler and I. C. Paul in *Polyether Antibiotics*, ed. J. W. Westley, Marcel Dekker, New York, 1982, vol. 2, ch. 3.

Paper 1/03848F
Received 26*th July* 1991
Accepted 16*th September* 1991