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Solution Conformation of Lasalocid and Lasalocid-Na^+ (X-537A)

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The complete unraveling of the proton nuclear magnetic resonance spectra at high field strength of Lasalocid (**1**) and its sodium salt (**2**) in different solvents allowed the definition of their solution conformations. The free acid, a lozenge-shaped molecule of 17 × 13 × 9 Å is prefolded in a way almost identical to that of its sodium derivative, where the ion lies in the centre, surrounded by all the oxygen atoms in the molecule except for the phenolic one. Although the upper side of the molecule is rather accessible to hydrophilic approach, some alkyl substituents are arranged out- and upwards, thus shielding the ion from possible contact with lipophilic surroundings. The spatial picture suggests that the ion is trapped upon arrival at one side (the flat upper side) and is released either at the same or at the opposite side.

INTRODUCTION

Lasalocid (**1**) is a relatively simple ionophoric antibiotic of the nigericin group (*1*) whose solid salt (absolute) conformation is known (*2*) but to our knowledge no direct information about its solution conformation has yet been obtained. The findings, as emerging from a ^1H nmr study at 300 Mc of the free acid and its sodium salt in different lipophilic solvents, are reported herein. Both $CDCl_3$ and C_6D_6 were used as the media; and, although the most important spectral data could be obtained through use of these solvents, the data for C_6D_6 solutions of the free acid and those for $CDCl_3$ solution for the sodium salt are the most complete. Comparison of the material in both solvents is thus possible and was necessary for reaching certain final conclusions.

RESULTS

There are only minor changes in the overall shape when a sodium ion is trapped, so that for the free acid the landing spot for the ion is rather limited. Only small rotations around some bonds are necessary in order to engulf the ion and screen it from the lipophilic surrounding. In Fig. 1 we display a photographic reproduction of an expanded model of the three-dimensional structure of the free acid molecule. The following stereochemical features, as will be commented on in the discussion section, are noteworthy. We follow hereby the numbering as depicted in representation **1**.

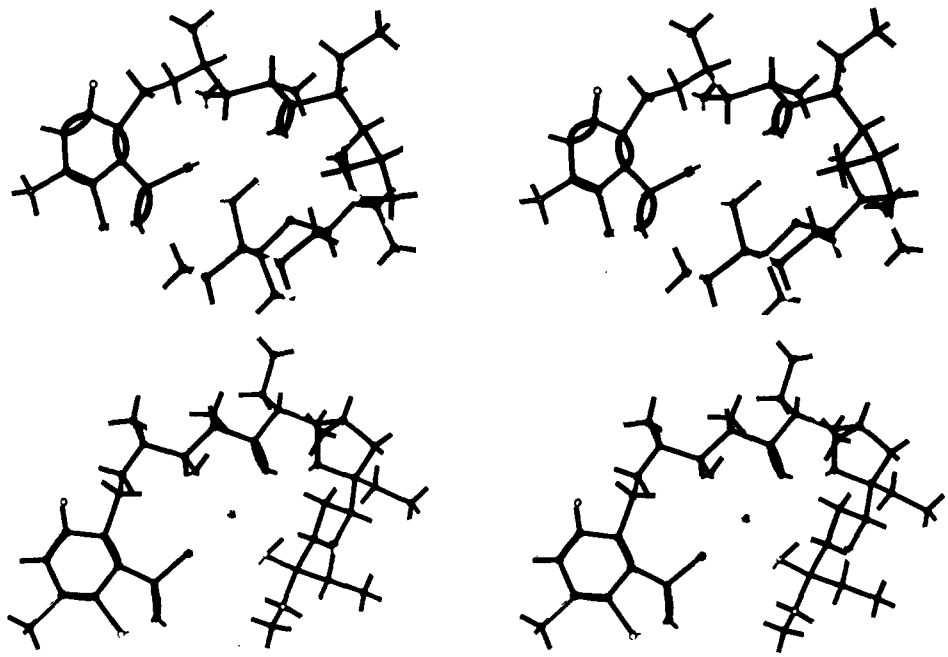
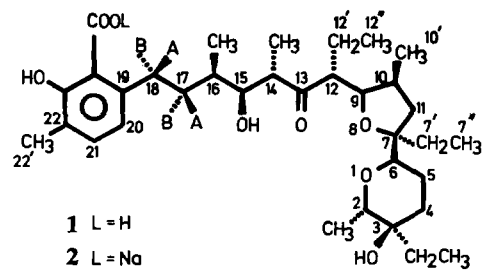


FIG. 1. (Top) Stereoscopic photograph of an expanded model of Lasalocid, free acid (**1**), viewed from the top of the molecule (direction of ion approach). (Bottom) Stereoscopic photograph of Lasalocid, sodium salt (**2**).

1. The plane of the aromatic nucleus bisects the bonds C_{17}–C_{18} and C_{18}–H; the carboxyl and hydroxyl substituents are thereby oriented towards the same side of the C_{16}–C_{15} bond (see **3**, **4**). The angle θ becomes somewhat smaller during the capture of an ion.

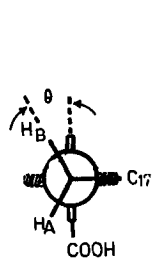


1 L = H
2 L = Na

2. The bonds C_{19}–C_{18} and C_{17}–C_{16} stay antiperiplanar (**4**), the projection angle τ (torsion) however being somewhat smaller than 60°, especially in the free acid **1**.

3. The C_{16}–H bond bisects the methylene protons around C_{17}–C_{16}; therefore C_{15} and C_{18} are syn clinical (gauche), as illustrated in **5**.

4. The rotameric disposition around C_{16}–C_{15} is characterized by a gauche CH/CH relationship (**6**), whereby in the free acid the angle is close to 90° while it is very nearly 90° in the sodium derivative.



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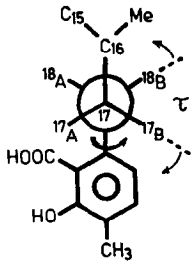

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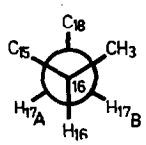

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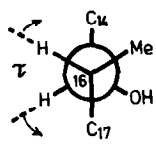
5. Around C_{15}–C_{14}, the carbonyl grouping stays gauche to OH-15 (with $\tau < 60°$; allowing H bridging).

6. Around C_{12}–C_9, two possible arrangements are consonant with the spectral characteristics, and we have represented in **8** the more probable one, as indicated by inspection of models and consideration of steric hindrance.



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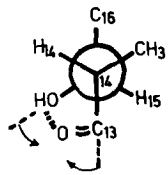

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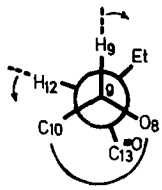

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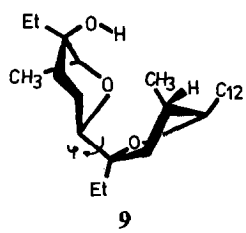
7. The tetrahydrofuran moiety occurs in a twist conformation having C_{10} above and C_{11} below the mean plane (a so-called south or S-form (*3*) or ${}^{10}_{11}$T-form (*4*)). Therefore the Me-10 group is pointing "upwards" (see **9**). We believe that this orientation prevents in the final molecular shape the approach of the hydrophobic surrounding towards the carbonyl and/or the cavity.



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8. The torsion ϕ around C_7–C_6 is most probably close to +60°.

9. The tetrahydropyran ring occurs in the expected chair form, with the bulkiest substituents in equatorial position but with OH-3 (and Me-2) in the axial position, the OH-3 being hydrogen bonded to O-1.

All these features allow one to propose the final conformation as depicted in the stereoscopic representation in Fig. 1, except that the rotational behaviour around the bonds C_{13}–C_{14}, C_{12}–C_{13}, and C_6–C_7 was not directly accessible from the spectral coupling parameters. A comparative solvent study and the relative shift values of H-5a and H-5e gave however quite convincing and unique information about these rotations, in accord with the final proposal; and we refer to the discussion section for further information.

Taking the slight spectral changes (e.g., coupling data) into account when passing from the free acid to the ion-captured species and as enumerated under features 1–9, one can easily tell from molecular models, such as represented by Fig. 1, that the ion must be trapped from the accessible (flat) top side, where one finds a relatively open region for hydrophilic approach towards most of the polar groups (e.g., OH groups, C=O, and salicylic part). Once trapped, slight rotations around C_{19}–C_{18} ($\theta<$), C_{18}–C_{17} ($\tau>$), C_{16}–C_{15} ($\tau>$) and presumably also around C_{17}–C_{16}, C_{14}–C_{13}, and C_{13}–C_{12} occur and result in a better screened and fenced cavity of ca. 3.5Å diameter, retaining the best access from the top. Departure of the ion via the opposite side, or bottom, of the molecule, however, cannot be excluded, especially because it would suffice to rotate the C_7–C_6 inter-ring bond (e.g., more or less concertedly with the rotation around the aromatic C_{19}–C_{18} bond) as to open the "back door" for release of the ion. These rotations should be relatively easy according to the presently proposed formation.

DISCUSSION

The spectra of Lasalocid, free acid, in C_6D_6 and of the sodium salt in $CDCl_3$ are displayed in Figs. 2 and 3, respectively, together with the assigned locations of most of the 54 protons. Spectral parameters are gathered in Tables 1 and 2. They have been extracted from repetitive and consecutive homo-INDOR and double irradiation (nmdr) measurements. Because of the complexity in overlap, no final refinements by simulated comparisons were attempted; and the observed and assigned absorption lines were interpreted in a first-order approximation. It is believed that, with some exceptions, the approximation is satisfactory because the most important spin systems lie separated enough from each other. This does not hold for the interactions H-10/H-11A/H-11B and H-5/H-4 in C_6D_6, and of the former set only the *sum* of coupling constants has been interpreted. Moreover the patterns for H-18B, H-16, H-10, H-11A, H-11B, H-12'B, H-5e, and H-4a were much too hidden (in C_6D_6) to permit extraction of individual coupling constant values. The more detailed discussion that follows refers to Lasalocid (**1**) in C_6D_6, but similar reasoning is applicable to the other cases. When necessary, the features which differ in other cases are explicitly mentioned. Some noticeable peculiarities of general character will first be treated.

The hydroxyl protons exchange with a relatively slow rate on the nmr time scale. Even the addition of 10 μl (ca. 1 equiv.) of acid did not narrow the hydroxyl absorption to the extent expected for free hydroxyl protons. However, nmdr applied at ca. 1.7 ppm did narrow the signals of H-15 and H-2. In this region no through-bond coupling partners could be located. The effect is brought up because (traces of) water absorb in the 1.7-ppm region. In irradiation experiments one also affects, via chemical exchange

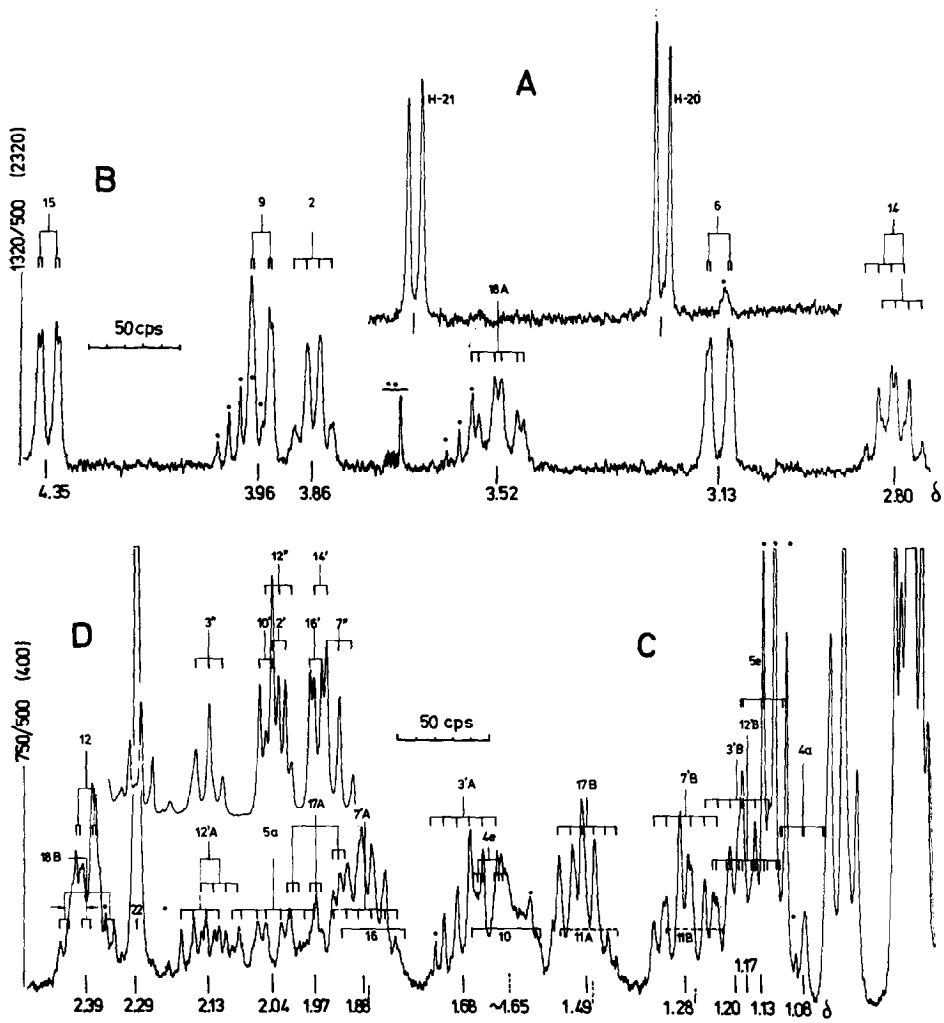

FIG. 2. Spectrum at 300 Mc (regions of 500 cps starting with a frequency value displayed at the left-hand margin of Lasalocid, free acid (1), in C_6D_6; TMS internal; sweep time, 500 sec. The assignments were made from first-order inspection, as followed from extensive homo-INDOR and double irradiation experiments (see Discussion section).

process, the hydroxyl protons. Therefore the couplings involving the latter (e.g., with H-15 and H-2) must disappear[1] (the OH-absorption band itself is too broad to allow complete saturation directly). It was independently shown that part of the observed broadening of the H-15 pattern (Fig. 2) remained after irradiation of H-16 [for which $J(15, 16) < 1.5$ cps was found], resulting in a residual small splitting (e.g. $J(15, OH)$). It follows that OH-15 is hydrogen bonded to the carbonyl, resulting (7) in

[1] The phenomenon has been described for phenolic and other mildly acidic hydroxyl protons (5). Although originally believed not to occur with aliphatic groups (5), we have previously made similar observations with tertiary alcoholic groups (6). We therefore believe that the phenomenon is general, provided that the appropriate exchange rates are in operation.

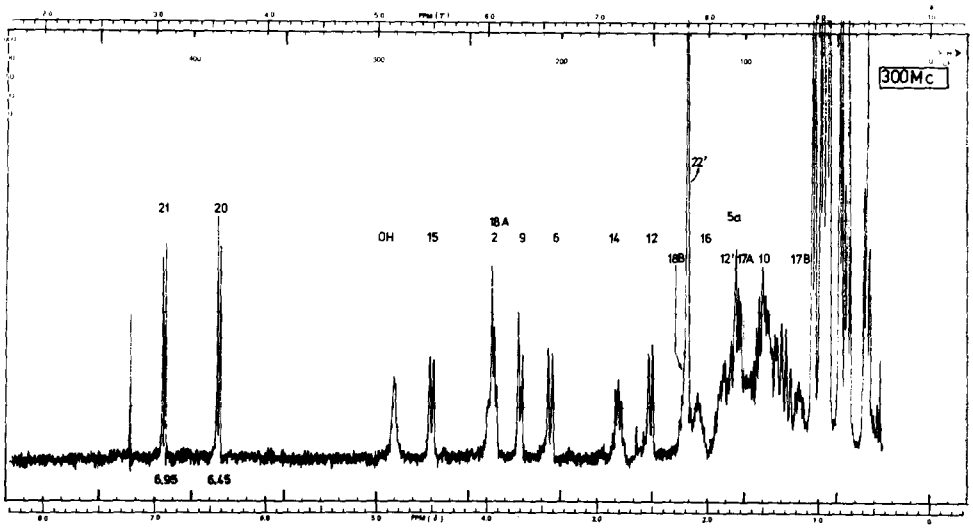

FIG. 3. Spectrum at 300 Mc of sodium Lasalocid in $CDCl_3$. Sweep width, 2500 cps; sweep time, 500 sec; TMS internal.

a small interproton coupling with H-15 (*gauche* relation). It is interesting to note the relatively more pronounced sharpness of the H-15 pattern in the sodium derivatives. Here the OH signals moreover are differentiated, one of these being relatively small (~10 cps in $CDCl_3$ and 8 cps in C_6D_6), showing up coupling. This relatively sharp OH signal is due to OH-3, as was ascertained by nmdr (H-2 quartet sharpening). The noticeable feature is that H-15 therefore couples less extensively (if at all) with OH-15; and from our final model it is indeed expected that the H bonding of OH-15 with C_{13}=O is largely lost while the carbonyl function is holding the ion in the centre of the molecule. The coupling involving H-2 then must be related to that of O*H*-3, although well-defined examples of couplings through four σ-bonds, are rare (*8*). However, the above-mentioned observations clearly reveal that both hydroxyl groups (OH-15 and OH-3) in **1** must be H bridged to a neighbour, as pictured in **9** and **7**. In the sodium derivative too, the appearance of a relatively sharp OH-signal, ascribed to OH-3 (nmdr) next to another much broader signal, indicates that in the present structure the conditions of exchange rate are in a range suitable for causing the observed phenomena. Intramolecular exchange however in this case is precluded by the presence of the central ion.

The assigned orientation taken up by the O_3–H bond through bridging with O-1 may be corroborated by the fact that only a small coupling exists between H-4a and H-5e. Although first-order analysis may fail in this case to give a correct value, it is certain that this coupling is smaller ($J < 1$ cps) than normally expected for a genuine chair conformation. The chair form of the tetrahydropyran ring is however corroborated by the findings that J(6a, 5a) = 12.0, J(5a, 4a) = 12.2 and J(5a, 4e) ~ 4 cps. Also J(6a, 5e) = 1.9 cps is smaller than expected. We have previously pointed out that the presence of a free oxygen orbital in a W-mode disposition (forming a planar zig-zag path) with respect to a coupling C–H bond renders the coupling with this proton smaller (*9*). Because H-5e (with respect to O-1) and H-4a (with respect to OH-3, if

TABLE 1

SHIFT DATA[a] OF PROTONS IN LASALOCID, (FREE ACID) (1), AND ITS SODIUM SALT (2) IN C_6D_6 AND $CDCl_3$[b]

Proton	2(e)	2′	3′A	3′B	3″	4e	4a	5e	5a	6(a)	7′A	7′B
$\delta(C_6D_6)$	3.86	0.87	1.68	1.20*	1.00*	1.68	1.08	1.13	2.04	3.13	1.88*	1.28*
$10^2 \times \Delta$(ASIS)[c]	+8	+22			−4					+35		
	+14	+25	−10	+17	−7			+33	−38	+43	−12	−12
$10^2 \times \Delta^+(C_6D_6)$	+24	+7										
$10^2 \times \Delta^+(CDCl_3)$	+12	+20	−20		−5	~−25		+6	−20	+32		

	7″	9	10	10′	11	12	12′A	12′B	12″	14	14′
$\delta(C_6D_6)$	0.76*	3.96	1.65	0.90•	~1.45• &~1.20•	2.39	2.13	1.17	0.87	2.80	⩽0.80
$10^2 \times \Delta$(ASIS)[c]	+6	−6		+13	+30 +30	+19			−4		+12
	+7	−12	+62	+14		+28	−25	+19	−3	+6	+17
$10^2 \times \Delta^+(C_6D_6)$	+6	+13	+7			−1				0	0
$10^2 \times \Delta^+(CDCl_3)$	0	−26	−5	−8	~−5 ~−5	+14	−20	+13	−30	+2	+2

	15	16	16′	17A	17B	18A	18B	20	21	22′	OH
$\delta(C_6D_6)$	4.35	1.87	⩾0.80	1.97	1.49•	3.52	2.39	6.56	7.01	2.29	6.1[d]
$10^2 \times \Delta$(ASIS)[c]	−24		+8			−15	+8	+5	+14	−7	5.27[e] & 3.33[f]
	−17	−12	+9	−20	0	−12	+6	+7	+17	−8	
$10^2 \times \Delta^+(C_6D_6)$	+33	−22	+9					+11	+7	+18	
$10^2 \times \Delta^+(CDCl_3)$	+17	−27	+19	−18	−19	+46	−16	−11	−6	−9	4.80[e]

[a] Values marked with an asterisk were only ascertained from homo-INDOR experiments and those with a filled circle only from nmdr; the others from both, and also mutually. Δ-values are to be added to $\delta(C_6D_6)$ in order to obtain the new value in ppm. This means that positive values represent upfield effects in benzene. Note that the sets 3′, 3″ and 7′, 7″ may be mutually exchanged.

[b] Shifts in ppm from TMS internal.

[c] The first row are the shift differences obtained in $CDCl_3$ solutions as such. The second row those after addition of ca. 1 equiv. trifluoracetic acid; whereby the resolution was slightly better and allowed additional assignments.

[d] Absolute value with $W\frac{1}{2} = 150$ cps.

[e] Id. with $W\frac{1}{2} \sim 15$ cps.

[f] Id. with $W\frac{1}{2} = 90$ cps.

suitably bridged to O-1, as proposed earlier) both have such W-arrangements, the observed values become fully rationalizable.

We will now discuss point by point the previously enumerated conformational features (see Results):

1. As the result of the well-known Barfield–Grant effect (*10*) and the observation that ^2J(18A, 18B) = 12.4 cps, the proposed orientation of the aromatic plane versus the methylene CH_2-18 group is settled. The fact that this value becomes even smaller in the salt **2** ($^2J \gtrsim 10.5$ cps) indicates a slight tendency for rotation of that plane in the direction toward an eclipsing with one of the C_{18}–H bonds (see arrow in **3**);

2. The antiperiplanar C_{19}–C_{18}/C_{17}–C_{16} arrangement follows from the observed vicinal couplings between protons 18 and 17 [the coupling of J(18B, 17B) was i.a.

TABLE 2

OBSERVED APPARENT COUPLING CONSTANTS (IN cps) IN LASALOCID (1) IN C_6D_6 AND SOME OTHER SOLVENTS FOR COMPARISON

J	2,2′	2,OH–3	3′A,3′B	3′,3″	4a,4e	4a,5a	4a,5e	4e,5a	4e,5e	5a,5e	5a,6a	5e,6a
1 in C_6D_6	6.7	~1.5	14.5	7.2	12.5	12.5	≤1	4	~2.5	13.0	12.0	1.9
1 in $CDCl_3$	6.8	~1.5	14.5	7.5				4.5		13.0	12.0	1.9
2 in C_6D_6	6.9	≥1.5		7.6		13.0		4.5		13.5	12.0	1.7

J	7′,7′	7′,7″	9,10	9,12	10,10′	Σ10,11	12,12′A	12,12′B	12′A,12′B
1 in C_6D_6	14.5	7.2	11.0	1.9	6.2	≥5	>10	?	14
1 in $CDCl_3$		7.2	10.8	1.8	6.2	≤5	9.5	~2.0	13
2 in C_6D_6	14.5	7.0	10.2	~0.5	6.2		11.2	10	

J	12′,12″	14,14′	14,15	15,16	15,OH-15	16,16′	Σ16,17	17A,17B	17A,18A
1 in C_6D_6	7.4	7.1	9.4	1.5		7.2	8	12.4	3.5
1 in $CDCl_3$	7.4	7.1	9.2	1.2		7.2	8	12.4	3.4
2 in C_6D_6	7.4	7.0	10.2	1.5	~2	6.8			—[a]

J	17A,18B	17B,18A	17B,18B	18A,18B	20,21	21,22′
1 in C_6D_6	12.5	12.4	~5	12.0	7.5	0.6
1 in $CDCl_3$	12.5	12.4	4	12.4	7.5	0.8
2 in C_6D_6	—[b]	—[a,b]	—[b]	—[a,b]	7.5	0.6

[a] ΣJ(18A) ca. 28 cps.
[b] ΣJ(18B) ca. 43 cps.

extracted through knowledge of the other individual couplings and the sum of 17B]. The assignment of A (lower field part) and B (absorption at higher field) may be rationalized by the same torsion, and, as indicated in **4**, H-18A and to a lesser extent also H-17A are the most deshielded partners because of the orientation of the carboxyl substituent,[2] which moreover stays planar with respect to the aromatic nucleus. Only a carboxylic moiety, pointing in the direction as shown, presents its deshielding cone[2] for H-18A and H-17A (*11*).

3. The sum of the coupling constants between H-16 and both H-17 does not exceed 8 cps, whence proposal **5**.

4. The coupling between H-15 and H-16, being small (<1.5 cps) (in the free acid) or nearly zero (in the sodium derivative), reflects their almost orthogonal disposition.

5. In fact this lack of coupling rendered the spectral connection between the pattern at 4.35 ppm (H-15) and a hidden one at 1.87 (H-16) most difficult, especially because small couplings between three other structurally analogous protons (H-12/H-9/H-10) with almost identically expected patterns of behaviour are also present. It was important to uncover the exact nature of H-10 because a relevant ASIS effect on

[2] No anisotropy calculations seem available for a carboxylic group with respect to its bond magnetic susceptibility and electric field contributions. However it is generally believed that carbonyl-containing moieties deshield the protons located near the nodal plane (cf. Ref. (*2*)). This would especially be true in the present case, where by virtue of H bond bridging and mesomerism the salicylic function forms one with the aromatic nucleus, thus extending the para- and diamagnetic regions of this nucleus towards the carboxylic and hydroxylic side.

H-10 (see below) was the only key for unlocking the rotational behaviour around C_{12}–C_{13} and C_{13}–C_{14} (orientation of the carbonyl group). The ASIS effect in C_6D_6 is so big that an inverse field order for H-16 versus H-10 is found in $CDCl_3$, rendering misleading comparison between the two solvent spectra, as criteria for assignment. We have each time tracked the correct assignments by consecutive INDOR and nmdr experiments starting from both H-18A and H-18B via both J-17A and H-17B, converging to H-16 on one side, and starting from both H-12′A and H-12′B via H-12 to H-9 (and H-10) on the other side.

6. Moreover, H-15 does sharpen (to a clearly defined doublet) while the thus identified H-16 is being irradiated, and the same holds for H-12 (collapse of fine splitting in the H-12 pattern while irradiating H-9). Therefore the torsion angle between the two protons H-9 and H-12 falls between 60° and 90°. There is an orientation possible other than the one illustrated in **8**, with the same angular relationship; but it is a very improbable one, because the carbonyl orientation would be outward, e.g., pointing into the unfriendly lipophilic medium. Furthermore the couplings J(12, 12′A) and J(12, 12′B) are relevant in this aspect. The ethyl-12 is not allowed to take equally the three possible rotameric situations as follows from widely different coupling values (9.5 and ~2 cps). Indeed, accepting that the carbonyl group points towards the cavity, then Et-12 becomes sterically hindered by Me-14; whence only one discrete rotameric situation is allowed. This is depicted in Fig. 1, and this choice is strengthened by the fact that it is the lowest field partner of H-12′ that shows up having the biggest interaction with H-12: H-12′A is deshielded by the pseudo-synaxial Me-14 disposition. This orientation certainly has something to do with keeping hydropholic surroundings away from the cavity.

We return to the problem of the carbonyl orientation.

7. The large coupling J(9, 10) and the relatively low sum of couplings J(10, 11A) + J(10, 11B) reveal that the preferred form of the tetrahydrofuran ring is the S-form ($^{10}_{11}$T; see **9**). This is (besides in other cases a N-form) one of the expected forms (*12*), the more so because the bulkiest groupings (e.g. C-12 and C-6) assume the quasi-equatorial positions, thus bringing the Me-10 group into a quasi-axial position. We have, in connection with our previously proposed C_{13}=O-inwards orientation, found welcome information about these facts in the study of ASIS effects on the several protons. The gross effect in substituting C_6D_6 for $CDCl_3$ is slight deshielding in the former solvent for most of the protons. There are however striking exceptions, namely, H-2, Me-2, H-12, H-6a, and H-10, the latter two being especially dramatic (0.43 and 0.62 ppm, respectively). It is known (*13*) that upfield effects occur at the positive ends of dipoles of a molecule, and this should be especially true in the C_2–C_{15} region if all the vector dipoles (C→O and C⇒O) are indeed oriented towards the cavity. Thus the observed ASIS effect corroborates firmly the proposed S-form (with H-10 directed outwards) and i.a. the C=O-inwards orientation.

8. Once we know the conformations of the tetrahydrofuran and tetrahydropyran rings (S-form and chair, respectively), it is possible to trace the rotameric behaviour around C_7–C_6 by inspecting shift values (Table 2). It is diagnostic that H-5a is found well *below* H-5e, contrary to the general observation that undisturbed axial ring protons resonate to the highest field position. The reason for the present reversal

becomes obvious by inspecting the model (cf. **9**). In that it is *syn* axial to OH-3, one expects a downfield displacement of H-5a; although the resulting magnitude would be at most 0.3–0.4 ppm (*14*). In the present case H-5a lies 0.9 ppm below H-5e. There must be other severely deshielding contributors in order to result in such a pronounced shift displacement (down to 2.03 ppm!). The picture that best explains this quantitatively results from the acceptance that ϕ of the C_7–C_6 junction is close to +60° (the origin being defined when both C–O bonds stay eclipsed,). One then finds H-5a and C_7–O_8 in a pseudo-*syn*-axial relation, and thus again shifts with ca. 0.3–0.4 ppm towards lower field.[3] Further contributions may result from the relative orientation of the C_{13}=O function (e.g., H-5a coming in the deshielding cone (*11*, *18*)), provided that this function indeed points inwards, as previously proposed.

Comparison between dissolved K^+-salt and crystal structure of $Ba(C_{34}H_{35}O_8)_2 \cdot H_2O$. The crystal structure of a hydrated Ba-salt has been reported (*2*). The picture in the solid state is altered by the fact that *two* molecules (otherwise not held together by any intercomplex association other than the ion) are not identically bound to the cation. The determination was therefore all the more complicated by the apparent pseudo-symmetry. On the basis of the published stereoscopic view (*2*) it is clear that in the solid state, both molecules, when coordinated to a divalent ion, are nevertheless remarkably similar in shape, the ion being surrounded by COO^--24, OH-3 in both units, as well as by O-1 and O-13 although through intermediation of a water molecule in (only) one of the units, and additionally by O-15 and O-8 of the other unit. Closer inspection reveals that the cation in the crystalline state is not in the middle of the backbone of one unit (as the Na^+ is in the solution conformation) but sandwiched between two units and, thus, out of the centre of both molecules. Therefore the conformation resembles surprisingly closely the free acid conformation in solution, that is, with the rotational differences around the bonds that change during salt formation previously reported in the present paper. As in solution (free and salt), an "end-to-tail" interaction between OH-3 and COO(H)-24 is clearly present, thus stabilizing each time the pseudo-cyclic conformations. The spatial resemblance between the free acid and its salt pinpoints the expected relative flexibility of the molecule, whereby only rotations around discrete bonds permit the molecule to adopt its shape readily to the kind of cation involved and whereby the direction of most oxygen atoms (e.g., O–1, O–8, and especially O–13 and O–15) is changed in a way as to surround with the appropriate distances and coordinates the cation that is presented to the antibiotic.

EXPERIMENTAL

Spectra were recorded at room temperature on a Varian HR-300, equipped with homo-INDOR and decoupling facilities (SC 8525-2). Lasalocid and its Na-salt were gifts of Hoffmann-Laroche (Basle, Switzerland), with respective preparation No.

[3] One might wonder why the normal order with respect to H-4a and H-4e is found, although H-4a suffers from a synaxial deshielding by Me-2 (*14–16*). The situation however is more complex. First, H-4a is *upfield* shifted by virtue of the vicinal antiperiplanar substituent (*15*). Second, the influence caused by the vicinal ethyl substitution, a nonspherical substitution, will depend on the rotameric population of the latter, for which we expect a relative slight deshielding of H-4e versus H-4a (*17*). Furthermore multiple effects are additive (*17*).

1804–120A-2 and 1804–116A-1 (Dr. J. W. Westley). About 50 mg was dissolved in the appropriate medium and kept in a refrigerator for several weeks. Slight spectral displacements were sometimes noticed which were not due to structural alterations but which were occasionally very helpful in the unraveling of extensive overlapping regions. Best resolutions were obtained for the free acid after addition of 10 μl of trifluoroacetic acid that caused a displacement of the common hydroxyl signal but only a relatively small narrowing of the broadened band ($W_{1/2} = 80$–150 cps). Care was taken during double resonance experiments to choose an optimal saturation strength, and the results of applying decreasing strengths were compared whenever necessary (ranging from 55 to 66 db for H_2 with 25 db for H_1). Homo-INDOR irradiations were invariably taken with 42 db for H_2. Difficulties were met while irradiating at positions corresponding to (the first) and second spinning side band positions of the strongest absorptions. To overcome this, different sample spinning speeds were applied whenever necessary.

Integrations of detailed regions were used for a rough division of proton groups in the spectrum, the total integration agreeing very well with the constitutional formula $C_{34}H_{54}O_8$ of Lasalocid.

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