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Solution Conformation of Monensin Free Acid, a Typical Representative of the Polyetherin Antibiotics

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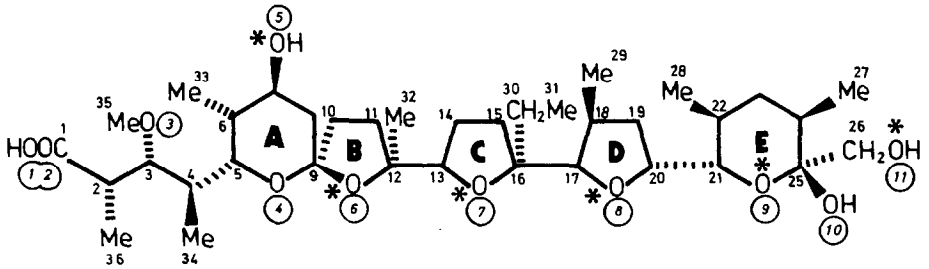
The solution conformation of the ionophore Monensin in its free-acid form bears a close resemblance to that of its Na^+ salt. The backbone is folded into a closed loop, and the pseudocyclic structure is shut by head-to-tail H bonding between the carboxylic function and the alcoholic functions of the last six-membered ring with the mediation of a water molecule. A mode of trapping is proposed and compared to features observed in some other membrane-active complexones.

INTRODUCTION

Monensin (**1**) is a typical representative of membrane-active antibiotics of the acid polyetherin family (*1*, *2*). A comparison of the three-dimensional folding between free and complexed ionophores provides a deeper insight into the mode of action of these polyetherins, viz., how cations are trapped in order to isolate them from their lipophilic surroundings and how they are transported across the membrane bilayer of cells. Monensin Na^+ salt (1^+) has recently been studied in solution (*3*). It typically adapts as a spherical pseudocyclic entity with a buttoning shut by H bonding between the head carboxylate and the end alcoholic functions OH-10 and OH-11. The sodium cation is trapped in a central cavity, coordinated by at least six oxygen atoms (see asterisks in the covalent structure **1**).

Some of the ionophores of this family offer a rather poor lipophilic-protecting shield for the trapped cation because they possess rather short backbone skeletons and



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because the circular form is flattened. This is the case for Lasalocid (*4*, *5*) and presumably also for Lysocellin (*6*), but it has been clearly shown (*5*) that this shortcoming disappears when dimeric complexes are formed. A dimer is also formed by A 23187–Mg^{2+} (*7*, *8*) in which a divalent cation is coordinated. However, such a dimerization does not seem to occur for the other members of the polyetherins such as Monensin.

The uncomplexed free-acid compounds mostly occur also in a circular conformation [e.g., Dianemycin (*9*, *10*) but not A 23187 (*7*)], and these forms differ only slightly from the complexed species. In this contribution we report our findings on the free-acid form of Monensin (**1**).

METHODS AND RESULTS

Figure 1 shows the ^1H nmr spectrum of **1** in $CDCl_3$ obtained at 300 MHz. The extracted parameters are collected in Tables 1 and 2 together with the earlier findings obtained for $\mathbf{1}^+$ (*3*). The parameters are obtained from first-order interpretation of the spectrum, where the patterns have been assigned through the use of consecutive double resonance (nmdr) experiments. Experimental methodology has been described previously (*4*, *7*, *11*). We were not able to extract detailed information about the protons H-10, H-11, H-15, and H-23.

The similarity, both in shift and coupling constant values, between **1** and $\mathbf{1}^+$ is striking. Differences in shifts (see Table 1, $\Delta^+ \cdot 10^2$) in general do not exceed 0.1 ppm with only a few exceptions. Opposite shift displacements for protons H-20 and H-21 or for protons H-26A and H-26B may easily originate from their different positions with respect to the central cation in $\mathbf{1}^+$, while the differences observed for some ring protons of the tetrahydrofurane moieties may arise in part from changes in the average of the several conformations that are possible for these rings in agreement with changes in interproton coupling values for **1** with $\mathbf{1}^+$.

The highly comparable coupling constant values (including those of the acyclic fragments) are noteworthy. Small changes probably arise around the bonds C_3–C_4, C_4–C_5, and C_{20}–C_{21} when comparing **1** with $\mathbf{1}^+$, but it is nevertheless obvious that the torsions around these bonds remain essentially the same. The geminal coupling $|^2J(26A, 26B)|$ is somewhat lower for **1**. It may result from the fact that the adjacent lone pair electrons on O-11 are in a somewhat better "parallel" disposition ["parallelity" effect, cf. ref. (*13*)].

The behavior of the hydroxylic absorptions are of particular interest and relevant to the detailed understanding of the spatial structure of **1**. There are three distinct patterns which belong to hydroxylic protons. All three are diminished on heating the sample and disappear entirely after treatment with D_2O (Fig. 1, spectrum B). They reappear with the same intensity (*vide infra*) when the sample is retreated with H_2O (e.g. Fig. 1, spectrum D). Irradiation at either of the three positions greatly decreases the intensity of the other remaining patterns, hence they do exchange chemically but at a rather slow rate, because even the approach of two of the patterns as close as <10 Hz (Fig. 1, spectrum A) does not result in a mutual collapse ($\tau > 10^{-2}$ sec). The doublet at δ 4.43 belongs to O*H*-5 where it is coupled to H-7 (nmdr; $J = 8.0$ Hz). We assign the peak at δ

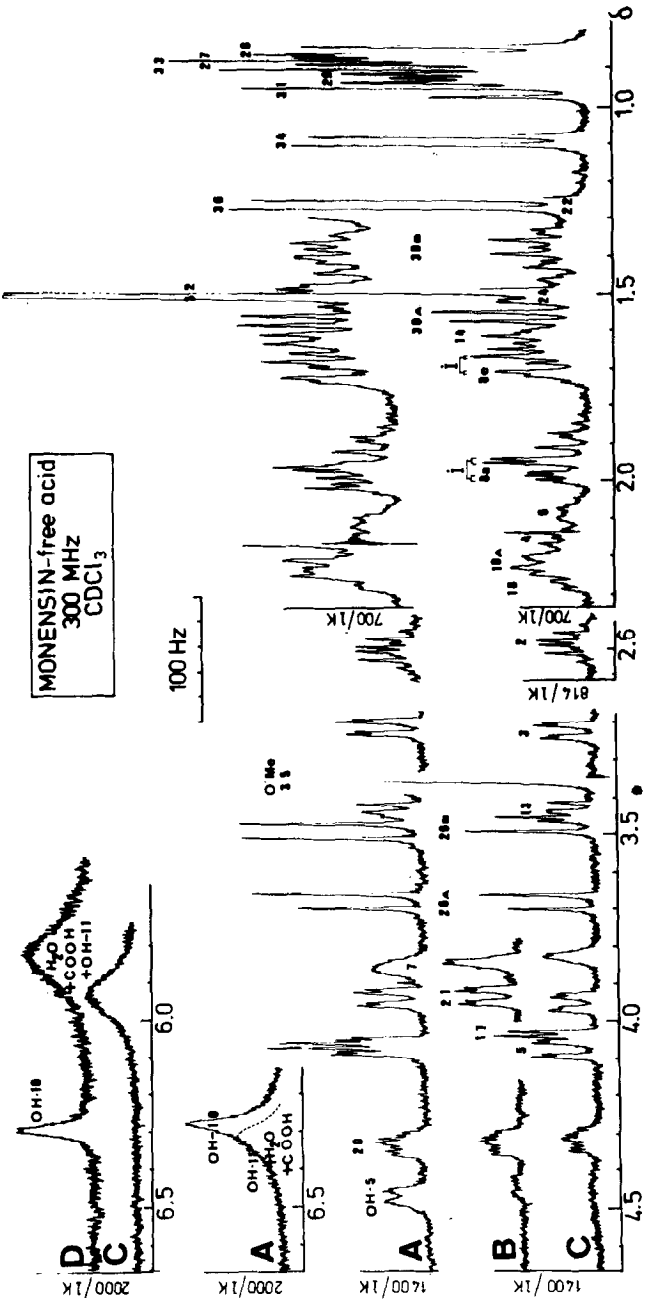

FIG. 1. 300-MHz ^1H nmr spectrum of Monensin free acid in $CDCl_3$. (A) Spectrum taken at 18°C. The OH patterns around δ 6.3 are more clearly resolved in a broad pattern flanked by a relatively sharp peak at slightly lower temperature (not shown); (B) spectrum at ca. 18°C after treatment of the sample with D_2O; (C) as in (A) but at ~50°C. Note in (B) and (C) the relative sharpening of the H-7 signal at $\delta \sim 3.8$ [$J(OH\text{-}5,7) = 8.0$ Hz].(D) After retreatment of the sample with H_2O; relative areas are: δ 6.3, one proton; δ(5.8/6.35), four protons; and δ 4.46, one proton.

TABLE 1

SHIFT VALUES (IN PARTS PER MILLION FROM TMS INTERNAL) OF PROTONS IN MONENSIN FREE ACID (1) AND MONENSIN Na^+ SALT (1^+) IN $CDCl_3$[a]

	Proton No.													
Compound	2	3	4	5	6	7	8a	8e	13	14A	14B	15A	17	18
1	2.61_6	3.21_6	2.18_3	4.07	2.16_7	3.86_0	1.98	1.70	3.45_0	~1.69	~1.57	1.9	4.09	2.23_3
1^+	2.53_6	3.18_9	2.06_2	4.03_5	2.24_7	3.89_0	1.91_5	1.69_0	3.54_3	1.76_2	~1.54	1.92	3.94_4	2.20
$\Delta^+ \cdot 10^{2b}$	−8	−2.7	−12	−3.5	8	3	−6.5	−1	9.3	~7	~3	~0	−14.5	−3
	19A	19B	20	21	22	24	26A	26B	Me-27	Me-28	Me-29	30A	30B	Me-31
1	2.20	1.38	4.33_3	3.94_0	1.27	1.50	3.67_8	3.49_6	0.87_7	0.85_6	0.92_7	1.55	1.37	0.95_8
1^+	2.20	1.53_5	4.39_7	3.82_8	1.48_5	1.38_3	3.98_0	3.29_7	0.80_5	0.85_0	0.93_7	1.55	1.50	0.94_3
$\Delta^+ \cdot 10^{2b}$	0	15.5	6.7	−11	21.5	−11.7	30.2	−20	−7	0	1	0	13	−1.5
	Me-32		Me-33		Me-34		OMe-35		Me-36		O*H*-5	O*H*-10		O*H*-11
1	1.49_6		0.89_0		1.09_9		3.36_7		1.25_9		4.46	~6.27		5.8/6.3[c]
1^+	1.50_9		0.89_6		1.17_9		3.38_0		1.23_9		3.55			9.60
$\Delta^+ \cdot 10^{2b}$	1		1		8		1		−2		−100			

[a] The data for 1^+ are taken from Ref. (1); for numbering, see structure 1.

[b] $\Delta^+ = \delta(1^+) - \delta(1)$.

[c] Variable position. This pattern integrates for four protons and is assigned to COOH-1 + OH-11 + H_2O (see text).

TABLE 2

APPARENT COUPLING CONSTANTS (IN HERTZ) OF 1 AND 1^+

Compound	2,3[a]	2,36	3,4	4,5	4,34	5,6	6,7	6,33	7,8a	7,8e	|8a,8e|	Σ13,14	
1	10.2	6.7	2.0	11.5	6.8	1.9	≳2	7.1	3.5	≳2	14.5	≲16	
$\mathbf{1}^+$	10.3	6.7	1.6	13.0	6.9	1.8	2.3	6.9	3.6	2	14.1	15.3	
	17,18	18,29	19A,20	19B,20	20,21	21,22	22,28	24,27	26A,26B	30A,30B	30,31	7,O*H*-5	24,O*H*-10
1	4.0	6.8	5.8	10.5	2.6	10.6	6.6	6.3	11.3	14.5	7.4	8.0	≲ 1.5[b]
$\mathbf{1}^+$	3.4	7.1	(7.0)	9.8	4.1	9.5	5.9	5.6	11.9	—	7.6	(1.5)	—

[a] For numbering, see structure **1**.

[b] From bandwidth consideration during {H-24} → OH-10.

6.27 to OH-10, because on irradiation of H-24 (δ 1.50) some narrowing of its bandwidth can be observed [J(24, OH-10) $\lesssim$ 1.5 Hz]. Integration reveals that the broad band at the variable position represents four protons, and even on shaking with an excess of water it retains this intensity. Hence next to the carboxylic proton and the remaining hydroxylic proton to be assigned (OH-11), a molecule of water is present in the structure of **1**. These protons, COO*H*-1, OH-11, and H_2O, appear to exchange chemically faster than the others. The assignment of COO*H*-1 and OH-11 as participating in the broad pattern is corroborated by the fact that (a) there is no other peak found in the spectrum (even as low as δ 20) and (b) both H-26 proton patterns are sharp doublets (^2J = 11.3) not involved in any other coupling, e.g., OH-11; the latter therefore takes part in a relatively fast exchange process [which was not the case in $\mathbf{1}^+$ (3)].

SPATIAL STRUCTURE OF MONENSIN

With the foregoing data in mind, it is possible to propose a detailed picture of the spatial structure of Monensin free acid. The final model must be pseudocyclic, similar to the salt $\mathbf{1}^+$ for which the conformation in solution (3) and in the solid state (12) is well established.

It is clear that the normal closure by head-to-tail hydrogen bridging is in the present case characterized by the participation of a water molecule. If we neglect the possibility of bifurcated hydrogen bonds (*14*) it is easy to propose from molecular model building the following hydrogen bonding scheme (Fig. 2): (i) OH-5 is the donor to O-11, itself

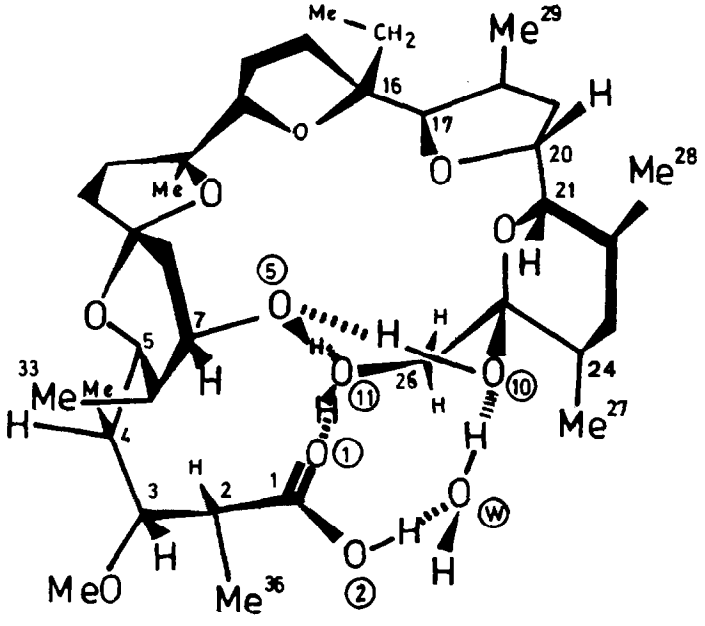

FIG. 2. Schematic drawing of a possible solution conformation in lipophilic solvents of Monensin in its pseudocyclic free-acid state, displaying the mediation of a water molecule in the head-to-tail buttoning shut as revealed by ^{1}H nmr spectroscopic data.

being the donor to O-1 of the carboxylic function; (ii) OH-10 is the donor to O-5; (iii) a molecule of water is sandwiched between O-10 (O_W is the donor) and OH-2 of the carboxylic function (O_W is the acceptor). This scheme would account for the features observed in the 300-MHz spectrum. First, rapid exchange between OH_W, COOH, and CH_2OH-11 seems logical; second, the orientation in space of OH-10 is such that the bonds $H–O_{10}–C_{25}–C_{24}–H_{24}$ form a planar zigzag pattern, and this would therefore account for the observed interaction [J(24,OH-10) $\lesssim$ 1.5 Hz];[2] third, the exchange possibilities for both OH-10 (bound to O-5) and OH-5 (bound to CH_2OH-11) would be less than for the remaining protons, where a water molecule is involved; and finally, perhaps the most relevant feature is the proper orientation of the O_5—H bond with respect to C_7—H, the torsion angle between the two bonds being $>130°$, resulting in a relatively large coupling, J(7, O*H*-5) = 8 Hz.[2] In this scheme also all the O—O distances that are bridged are less than 3 Å, and may result in moderate to strong bonds (*15*). Further, all the valence angles CO—(H)—O are close to 120°, except for $C_{25}O_{10}–O_5$ which is smaller (from rigid model constructions this would be ca. 110°). However, relaxation of the local deformation may allow even a better disposition for the latter.

We have represented schematically the cyclic conformation of **1** in Fig. 2. However, earlier findings by X-ray analysis (*16*) for the solid offer another possibility. Here the conformation would again be very similar to the salt (torsion angles differing by not more than $\lesssim 20°$) with a water molecule occupying the position of the cation and therefore located in the inner cavity, efficiently screened from the surroundings. The features in spatial orientation of OH-5 and OH-11 are comparable to the model depicted in Fig. 2, and it is therefore difficult to decide on mere ^{1}H nmr findings what hydrogen bond scheme is actually present in solution. The fact that the protons of the clathrated water exchange only very slowly even under "wet conditions" is perhaps in favor of the data obtained in the solid by Lutz *et al.* (*16*). The present study reveals that the acid ionophores possess a solution conformation that is only slightly different from their salts. There is relatively little information available for the free forms from solid state studies, but what is available also reveals this peculiarity [e.g., Lasalocid, Ref. (*17*)]. There is no indication that the ion would be trapped by a "pincers-mechanism," and the final form necessary for ion transport is in most cases already premolded in the free carrier. A simple replacement of a water molecule by the ion, with slight adjustments of the coordinating ligands and accompanied by small skeleton torsions, allows the molecule to pass from its resting into its active form. Such limited changes may account for a rapid process as during the transition period the solvation of the ion may be partly taken over by the ionophoric water molecule.

Presumably the presence of a water molecule is not a prerequisite. In Dianemycin, for instance, it is not clear if water is participating in the solution conformation of the free acid (*10*), whereas in Lasalocid in the crystal (*17*) it does. It seems that water is especially efficient for realizing the circular form if relatively short chains are involved, where it might be efficient in helping the head-to-tail closure and mimic the presence of a cation otherwise present in the pocket thus formed.

[2] This is a minimum value because the O*H*-10 partner is involved in some exchange (broadening); this tends to result in a decreased apparent coupling value.

During trapping, the rotation around C_1–C_2 may be the most typical, as this bond is revealed (from X-ray studies of the several members of this series) to assume easily slightly different rotational positions. Thus the carboxylate group participates directly in coordination in some of the polyethers [e.g., Lenoremycin–Na^+ (*10*, *18*), Nigericin–Na^+ (*19*), [Lasalocid]$_2$–Ba^{2+} (*20*), and A 204A–Na^+ (*11*, *21*)], but in others it does not and is turned away from the central cavity [e.g., Monensin–Na^+ (*3*), Dianemycin–Na^+ (*9*, *10*)]. Then it serves only to constrain the molecule in a loop and to keep the entity electronically neutral. It is therefore conceivable that the rotation around the C_2–C_1 OOH- rotor is the most important feature both for the final conformation and during trapping. In more complex ionophores, the trapping may be characterized by a somewhat more sophisticated event. Lenoremycin (=Ro 21-6150), Dianemycin, Septamycin, A204A, and Etheromycin (=CP 38295) are all characterized by a very similar backbone skeleton but bear an additional sugar-like fragment attached to it at different positions (and different absolute configurations). It has been proposed (*10*) that in Lenoremycin, but not in Dianemycin, the trapping might be accompanied by a rotation around the bond to which this extra fragment is attached, whereby the latter covers the cavity in the final stage of the ionic encapsulation. The reason for the presence of the sugar fragment in Dianemycin is less obvious, as it does not participate in ligand formation. It is perhaps a more primitive expression of a molecule where its presence only serves as an addressing entity for recognition of the site of action.

In general, however, the capture of ions in the present ionophores is as simple as for the genuine cyclic structures, such as Nonactin (*22*), although for Lasalocid an entropically less favorable dimerization to higher ordered complexes seems necessary (*5*).

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