Conformation in Solution of Carboxylic Ionophore Cationomycin: Acid Form and Li^+, Na^+, K^+, Rb^+, Cs^+, NMe_4^+ Complexes

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NMR study of the acid form and Li^+, Na^+, K^+, Rb^+, Cs^+ and NMe_4^+ neutral 1:1 complexes of the title compound was carried out in $CDCl_3$ and CD_3OD. Comparison was made with the corresponding species obtained with monensin a well-known related carboxylic ionophore. Resonance assignments were made by 2D NMR methods and nuclear Overhauser effects were measured by ROESY experiments. In $CDCl_3$, postulated conformations are very similar for the two ionophores; only local differences were observed between the acid and the complexes. In CD_3OD the monensin free acid presents a more open structure. Very small differences were recorded for cationomycin in the two solvents, this unusual behaviour may be related to the presence of the aromatic ester moiety. The different behaviour observed between the two bacterial ionophores cannot explain by itself the pronounced selectivity in binding for Na^+ (monensin) and K^+ (cationomycin).

Carboxylic polyether antibiotics are known for their ability to form complexes with cations and transport them through biological membranes;[1] they constitute a large class of naturally occurring ionophores, mostly isolated from Streptomycetacea. However, cationomycin[2] **1a** examined in this work was isolated from *Actinomodura azurea* of the Nocardiacae family. It displays selectivity of complexation and transport[3] for K^+ and shows a surprisingly low toxicity in mice[2] compared with other ionophores of the group. Its molecular structure closely resembles that of monensin **2a**; similarities and differences can be seen in Fig. 1. Interestingly, monensin is selective for Na^+[4] in model membrane systems. Apart from a partial assignment of its ^{13}C NMR spectra,[5] no conformational study has been performed on cationomycin. We report here a comparative study of conformations in solution of cationomycin, monensin, and their complexes with Li^+, Na^+, K^+, Rb^+, Cs^+ and NMe_4^+ using NMR in $CDCl_3$ and CD_3OD. This enabled us to test for a relationship between conformation and selectivity. To our knowledge, this is the first systematic investigation in the alkaline series of neutral complexes of polyether antibiotics. We also compare results obtained in solution with structures described in the solid state.

Results and Discussion

NMR Data.—^1H and ^{13}C resonance assignments were obtained by 1D and 2D NMR methods, ^1H–^1H chemical shift correlations (COSY,[6] COSY–DQF[7]), ^1H–^{13}C chemical shift correlations, and ^1H–^{13}C long range chemical shift correlations,[6] following a procedure previously described.[8] We give here only ^1H and ^{13}C chemical shifts corresponding to cationomycin and its complexes in $CDCl_3$ (Tables 1 and 2). The values for monensin are not given.[9] We have completed and corroborated those obtained by Anteunis[10,11] (^1H) and Ajaz *et al.*[12] (^{13}C) for the free acid and the sodium complex.

Nuclear Overhauser effects (NOEs) were recorded by ROESY experiments in the rotating frame.[13,14] No measurable NOE effects could be obtained by classical methods for ionophore antibiotics in the 200–400 MHz resonance range. A limited RF field strength for the mixing period in the ROESY experiment is beneficial for the suppression of spurious resonances due to COSY and Hartmann–Hahn type transfers.[13,15] The chemical shift differences in the cationomycin and monensin sets are weak, so the offset dependance will be the same for the two molecules and it is possible to compare the correlation peak intensities.[16,17] NOEs are classified into strong, medium and weak peak intensities.[18,19] The attribution of ^1H *pro R* and ^1H *pro S* of methylenes, the respective position of the heterocycles and the position of the ring protons are effected by means of these NOE effects.

^1H–^1H coupling constants were obtained directly from 1D ^1H spectra through double resonance experiments, and from 2D δ–δ ^1H–^1H and 2D J–δ ^1H–^1H correlations. Values of coupling constants for cationomycin and its complexes in $CDCl_3$ are given in Table 3; those corresponding to monensin are not mentioned.[9] Scalar and space couplings enabled us to determine the ionophore conformation.

Conformation of Cationomycin in the Acid Form; Comparison with Monensin.—*In* $CDCl_3$. Tetrahydropyran rings A in both ionophores and E in monensin adopt a chair conformation. For the ring A, as coupling constants $J_{7\text{-}8A}$ and $J_{7\text{-}8B}$ are lower than 4.2 Hz, 7-H is equatorial, 33-Me or 34-Me, 7-OH, 5-H are axial. The C-5–C-4 is equatorial with 4-H and 5-H antiperiplanar ($J_{4\text{-}5}$ ~ 11–11.5 Hz) (Fig. 2*a*). For the ring B, it can be deduced from $J_{11B\text{-}10A}$ and $J_{11B\text{-}10B}$ (1 and 3.5 Hz) that 11B-H is pseudoequatorial and from $J_{10A\text{-}11}$ (~ 4 and 10 Hz) that 10A-H is pseudoaxial. Ring B conformation in both ionophores would correspond to an envelope with C-10 at the top (Fig. 2*b*). The ring C is different in the two ionophores. For monensin, 13-H–14A-H and 14A-H–15A-H are antiperiplanar from the coupling constant values (respectively 10.8 and 9.0 Hz). The tetrahydrofuran ring C of monensin therefore occurs in a 14–15 twist conformation (Fig. 2*c*). For cationomycin, 13-H and 14B-H are antiperiplanar ($J_{13\text{-}14B}$ = 11.6 Hz). The NOE between 15-H and 14B-H, 30-Me and 15-H, the coupling constant values $J_{15\text{-}14A}$ ~ 0 Hz and $J_{15\text{-}14B}$ = 6.5 Hz can be explained if 15-H is equatorial, the ring C of cationomycin corresponding to an envelope with C-14 at the top (Fig. 2*d*). For ring D of cationomycin, the NOE between 17-H and 19A-H and the coupling constant values $J_{17\text{-}18}$ = 4.1 Hz, $J_{20\text{-}19A}$ = 6.5 Hz and $J_{20\text{-}19B}$ = 8.5 Hz can be explained by an envelope conformation with C-18 at the top. For monensin, a similar conformation is deduced from equivalent NMR data (Fig. 2*e*). In ring E, 21-H is antiperiplanar to 22-H from the coupling constant values $J_{21\text{-}22}$ ~ 10 Hz, consequently and including NOE it is deduced that ring E of monensin has a chair conformation with 28-Me, 27-Me and CH_2OH equatorial

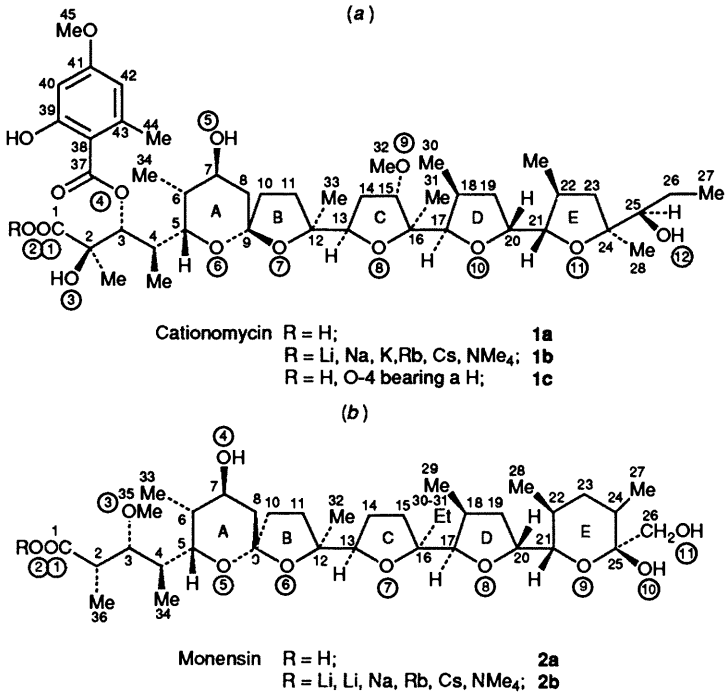

Fig. 1 Cationomycin **1a** (R = H); salts **1b** (R = Li, Na, K, Rb, Cs, NMe_4); derivative **1c** 4-(O)-H and monensin **2a** (R = H); salts **2b** (R = Li, Na, K, Rb, Cs, NMe_4)

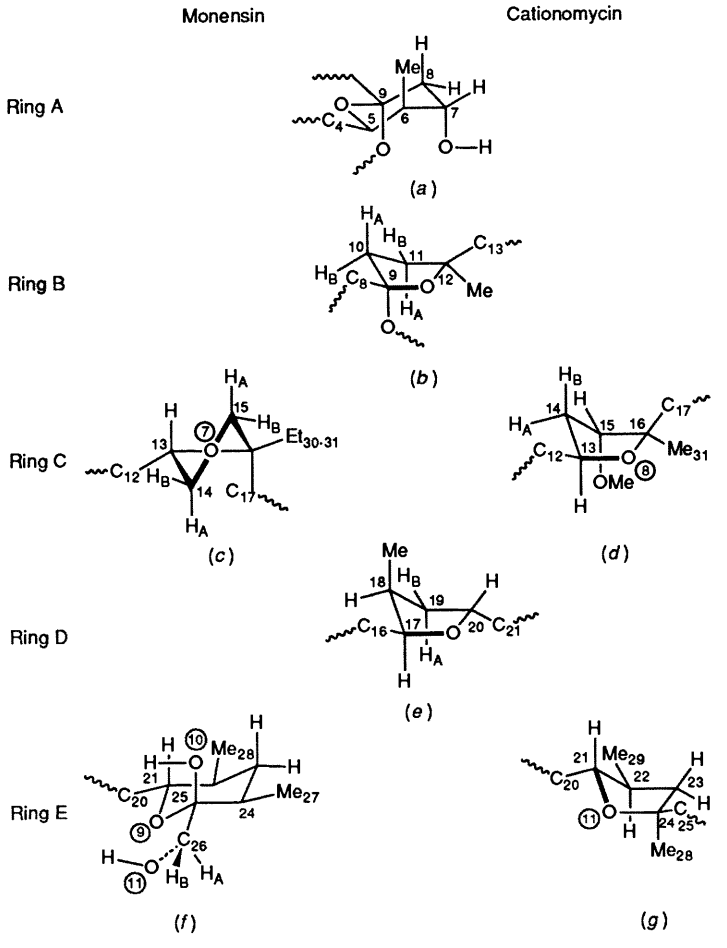

Fig. 2 Monensin and cationomycin acid forms: conformation postulated for each heterocycle

(Fig. 2*f*), and that ring E of cationomycin has an envelope conformation with C-21 at the top (Fig. 2*g*).

From values of coupling constants J_{3-4} ~ 2.5 Hz, J_{4-5} ~ 11 Hz, J_{20-21} ~ 2.8 Hz, and from NOE, it can be deduced that rotors C-3–C-4, C-4–C-5, C-12–C-13, C-16–C-17, C-20–C-21 of monensin and cationomycin are in identical fixed positions with

Table 1 ^{1}H Chemical shifts in $CDCl_3$[a]

^{1}H	Catio. ac.	Catio.-NMe_4	Catio.-Li	Catio.-Na	Catio.-K	Catio.-Rb	Catio.-Cs
3	5.61	5.59	5.61	5.65	5.57	5.53	5.55
4	2.04	2.12	2.07	2.14	2.24	2.14	2.07
5	4.08	4.10	4.13	4.17	4.16	3.94	3.83
6	2.51	2.45	2.50	2.48	2.43	2.43	2.45
7	3.88	3.85	3.90	3.90	3.85	3.84	3.84
8A	1.91	1.91	1.95	1.91	1.86	1.88	1.90
8B	1.59	1.60	1.60	1.60	1.70	1.71	1.53
10A	2.19	1.94	1.96	1.97	2.00	1.94	1.94
10B	1.55	1.71	1.69	1.71	1.68	1.67	1.67
11A	1.92	1.92	1.96	1.96	2.00	1.94	1.99
11B	1.65	1.62	1.64	1.59	1.54	1.64	1.56
13	3.62	3.59	3.64	3.64	3.60	3.59	3.59
14A	1.77	1.77	1.78	1.79	1.68	1.69	1.73
14B	1.53	1.54	1.55	1.57	1.58	1.69	1.61
15	3.93	3.91	3.94	3.95	3.96	3.96	3.92
17	3.75	3.77	3.78	3.80	3.67	3.65	3.73
18	2.38	2.35	2.39	2.39	2.34	2.34	2.38
19A	2.20	2.22	2.24	2.24	2.21	2.10	2.23
19B	1.56	1.56	1.60	1.58	1.53	1.48	1.49
20	4.26	4.24	4.27	4.28	4.30	4.28	4.26
21	4.03	4.01	4.02	4.04	3.81	3.82	3.97
22	1.83	1.83	1.81	1.87	1.72	1.71	1.75
23A	1.94	2.00	1.97	1.97	1.96	1.96	1.96
23B	1.56	1.44	1.58	1.58	1.63	1.55	1.58
25	3.84	3.80	3.73	3.81	3.55	3.51	3.70
26A	1.51	1.44	1.44	1.45	1.38	1.38	1.41
26B	1.14	1.07	1.15	1.12	1.12	1.14	1.13
27	0.94	0.93	0.97	0.95	0.90	0.88	0.93
28	1.11	1.09	1.12	1.13	1.13	1.10	1.10
29	0.97	0.96	1.03	1.00	0.99	0.96	0.98
30	0.99	0.96	0.99	0.98	0.96	0.95	0.97
31	1.13	1.10	1.14	1.14	1.11	1.10	1.12
32	3.28	3.27	3.27	3.27	3.25	3.24	3.26
33	1.51	1.21	1.52	1.54	1.51	1.45	1.42
34	0.95	0.92	0.98	0.93	0.97	0.97	0.96
35	1.30	1.27	1.31	1.30	1.28	1.30	1.26
36	1.29	1.27	1.31	1.32	1.31	1.10	1.32
40	6.27	6.28	6.29	6.30	6.31	6.27	6.18
42	6.31	6.25	6.32	6.33	6.35	6.32	6.26
44	2.68	2.67	2.70	2.71	2.72	2.60	2.61
45	3.77	3.77	3.78	3.80	3.79	3.77	3.75

[a] For methylenes A and B refer to the proton at lower and higher field site.

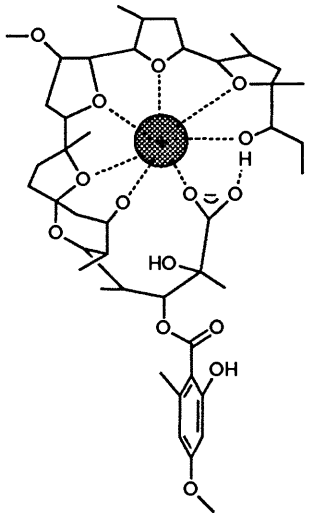

Fig. 3 Postulated structure for 1:1 cationic complexes with cationomycin

dihedral angles of respectively 70° for 3-H–4-H, 180° for 4-H–5-H, 50° for 20-H–21-H. The rotor C-2–C-3 is also in a fixed position, with a dihedral angle of 180° for 2-H–3-H in the case of monensin (J_{2-3} = 10 Hz) and a similar value for 3-OH in the case of cationomycin (NOE). The 25-OH in the cationomycin has δ 8.2 corresponding to a hydrogen bond 12-OH → 2-O between this group and the carboxylic function.

The rings and rotors of the two molecules being in preferential positions, it is possible to describe with a high precision the conformation in solution with head-to-tail buttoning OH → CO_2H, as in Fig. 3 for the complexes (interaction OH → COO^-). The conformational differences between cationomycin and monensin are slight, both molecules have a fairly stiff preformed cavity which can receive an ion. However, in cationomycin there is an aromatic ester group which stands out orthogonally to the cavity and in monensin double buttoning is possible by chelation between the hemiacetal ring and CO_2H (11-OH → 1-O and 2-OH → 10-O).

In CD_3OD. NMR study of ionophore systems in a protic solvent such as methanol affords a better understanding of the cation complexation–decomplexation mechanism at the water–membrane interface. Monensin shows significant conformational variations on changing from $CDCl_3$ to CD_3OD, while cationomycin exhibits a very similar structure in both solvents. Thus, the J_3 ^{1}H–^{1}H coupling constant for the acyclic parts of the monensin skeleton suggests a mobile open structure, with the head-to-tail chelations removed.[19,20] Conversely, for cationomycin there are only very small differences for ^{1}H and ^{13}C chemical shifts and proton coupling constants between

Table 2 ^{13}C Chemical shifts in $CDCl_3$

^{13}C	Catio. ac.	Catio.-NMe_4	Catio.-Li	Catio.-Na	Catio.-K	Catio.-Rb	Catio.-Cs
1	177.64	178.72	178.77	178.79	180.45	179.55	178.97
2	76.28	76.67	76.68	76.68	76.66	77.10	76.40
3	77.09	77.30	77.30	77.52	77.10	76.56	77.30
4	40.16	39.54	39.79	39.49	38.81	39.58	42.18
5	68.31	67.88	67.96	67.50	67.07	68.14	69.09
6	34.54	34.93	34.90	34.96	35.43	35.11	35.19
7	70.23	70.68	70.43	70.55	70.88	70.33	70.26
8	33.64	33.95	33.95	33.85	33.98	34.21	34.07
9	107.01	107.44	107.53	107.48	108.04	108.00	107.88
10	32.82	33.39	33.57	33.53	33.98	33.83	32.93
11	39.12	39.22	39.28	39.17	38.91	39.18	39.42
12	85.03	85.05	85.14	85.06	84.97	85.05	85.21
13	80.75	80.86	80.91	80.95	80.88	80.62	80.85
14	33.36	33.26	33.12	33.08	32.75	32.52	32.93
15	80.80	80.86	80.91	80.95	80.54	80.41	81.01
16	86.94	87.10	87.12	87.12	86.53	86.43	86.99
17	86.76	86.73	86.84	86.91	86.02	85.62	85.43
18	35.09	35.33	35.29	35.23	35.78	35.59	35.50
19	33.03	29.31	33.00	33.08	32.11	31.62	32.16
20	77.75	78.11	78.04	78.06	77.49	77.45	78.07
21	85.41	85.68	85.79	85.64	86.31	85.62	85.87
22	34.54	34.93	34.90	34.96	35.22	35.11	34.65
23	38.59	39.22	38.84	38.79	39.22	38.68	38.93
24	85.83	86.01	86.03	86.20	85.21	85.05	85.57
25	75.21	75.39	77.30	75.46	77.10	76.86	73.25
26	27.17	27.29	27.25	27.39	26.83	26.44	26.77
27	10.02	10.81	10.61	10.64	10.47	10.03	10.54
28	25.69	25.64	25.85	25.84	25.57	25.56	26.37
29	15.53	15.83	15.44	15.76	14.91	14.72	15.42
30	13.52	13.87	13.82	13.82	13.55	13.34	13.89
31	17.79	17.72	17.89	17.95	17.71	17.48	17.81
32	57.42	57.61	57.59	57.62	57.61	57.42	57.59
33	27.28	27.44	27.49	27.52	27.73	27.48	27.39
34	10.36	10.46	10.30	10.28	10.05	9.80	10.06
35	13.24	13.18	13.09	12.91	13.23	13.34	12.04
36	26.68	26.79	26.84	26.81	26.49	25.56	27.16
37	170.31	170.46	170.47	170.73	170.97	170.26	170.49
38	105.84	107.44	106.95	105.96	105.86	108.00	106.13
39	144.07	144.03	144.23	144.13	144.17	143.89	144.26
40	110.64	110.78	110.69	110.92	111.01	110.14	110.78
41	165.29	165.26	165.89	165.70	165.85	165.80	165.25
42	98.94	99.13	99.17	98.99	99.00	98.97	100.01
43	163.85	163.63	163.71	163.77	163.87	163.49	163.22
44	24.70	24.78	25.01	24.96	24.96	24.50	25.89
45	55.05	55.19	55.18	55.28	55.28	54.98	55.06

$CDCl_3$ and CD_3OD.[9] For instance, variations are 3.0–3.5 Hz for J_{3-4}, 11.0–10.5 for J_{4-5}, 2.7–1.0 for J_{20-21}. In this last case 1 Hz corresponds to a dihedral angle of approximately 90° (70° in $CDCl_3$). Rotors and cycles in the cationomycin molecule stay in very similar fixed positions in both solvents (Figs. 2, 3). It even seems likely that the 12-OH → 2-O is not suppressed in CD_3OD, the aromatic ring in the vicinity of the carboxylic group causing steric hindrance to solvation.

Conformation of Complexes.—Cationomycin. The ^1H and ^{13}C chemical shifts in $CDCl_3$ for Li^+, Na^+, K^+, Rb^+, Cs^+ and NMe_4^+ neutral complexes are not very different in comparison with cationomycin acid. It is important to note that the tetramethylammonium ion is too voluminous to be accommodated in the ionophore cavity; we thus have access to the free anion structure by NMR spectroscopy, the cation being exterior to the cavity. From the variations of the chemical shifts, it appears that the ends of the molecule and cycles A, B are most affected by the cation complexation. No systematic variations were recorded and no strict correlation could be found according to the ionic radius of the complexed cation.[9] Only 7% of the protons showed a chemical shift evolving linearly with the ionic radius. Using the covalent radius for the ions[21] did not enhance correlation. Coupling constants of the complexes could also be compared with the acid form: systematic variations are observed only for J_{7-8A}, J_{7-8B}, J_{20-19B} and J_{25-26A}, coupling constants for the complexes are globally identical to that of the acid form, indicating a close similarity between the globular conformations of all the skeletons. From all the data obtained by NMR the following structure for the complexes in solution can be postulated: 1-, 6-, 7-, 8-, 10-, 11- and 12-O are the cation coordinating sites, the complexing cavity is stabilised by 12-OH → 2-O interaction (Fig. 3); this conformation is very close to that described in the solid state for the thallium salt.[22] The selectivity of cationomycin for potassium is not apparent from the NMR results.

In methanol and in chloroform the conformations of the cationomycin complexes are identical; this observation had already been made for grisorixin–potassium[23] and for the monensin alkaline complexes.[24] This is also true for cationomycin–NMe_4^+ where the big ammonium cation is out of the anionic cavity; in this case the NMR parameters and especially the coupling constants of the rotors are also globally identical, 3 Hz for J_{3-4}, 11 Hz and 10 Hz for J_{4-5}, 2.0 and 2.2 Hz for J_{20-21}, respectively in $CDCl_3$ and CD_3OD. This last result can be explained by the presence of the aromatic ring which, like in the acid form, causes steric hindrance to solvation in the region of the head-to-tail interaction.

Table 3 Coupling constants in $CDCl_3$

J/Hz	Catio. ac.	Catio.-NMe_4	Catio.-Li	Catio.-Na	Catio.-K	Catio.-Rb	Catio.-Cs
3–4	3.0	3.0	3.0	3.0	3.5	3.0	3.0
4–5	11.0	11.5	11.5	11.0	12.0	11.0	11.5
4–35	7.2	7.0	7.0	7.0	7.2	7.5	7.0
5a–6e	2.0	1.0		2.0		1–1.5	1.5
6e–34	6.8	7.5	6.5	6.5	6.5	6.5	
6e–7e	2.0	1.5		2.0		2.5	
7e–8Ae	2.0	3.0	3.0	3.0	4.0	3.5	
7e–8Ba	2.0	6.0	5.0	6.0		6.0	6.5
8Ae–8Ba		14.0	14.0	14.0		14.0	12.0
11A–11B	13.0						
11B–10	1–3.5						
13–14A	4.1	4.0	4.0	4.0	4.0	4.5	4.0
13–14B	11.6	11.5	11.0	11.5	11.5	10.5	10.5
15–14A	< 1	< 1	< 1	< 1	< 1		
15–14B	6.5	6.5	6.5	6.5	7.0		
14A–14B	12.5	12.5	12.5	12.5			
17–18	4.1		4.0	4.0	4.0	3.5	4.0
18–30	6.5	7.5	6.5	7.0	6.5	6.5	6.5
18–19A			4.5	4.3		4.5	
18–19B					1.0		
19A–19B		10.5	10.5	11.5		10.0	
20–19A	6.5	6.0	6.5	6.5	7.0	5.5	5.5
20–19B	8.5	10.0	10.0	10.0		10.5	10.0
20–21	2.7	2.0	2.0	2.5	2.2	1–1.5	1.5
21–22	10.0	10.5	10.0	10.5		10.5	10.5
22–29	6.1	7.5	7.0	6.0		6.0	6.5
23A–23B		9.0	10.0	10.1		9.0	
25–26A	9.9	2.0		2.0	10.0	2.0	9.0
25–26B	2.0			10.0	< 1	1.0	2.0
26A–26B		13.5	13.5	13.0		14.0	14.0
27–26	7.5	7.5	6.5	7.5	7.0	7.0	7.0
40–42		2.5		2.8		2–2.5	2.5

We studied the cationomycin skeleton without its aromatic arm in the 3-position, as it has been shown that this derivative **1c** obtained by simple ester hydrolysis presents modified ionophore and antibiotic properties in comparison with the natural metabolite.[3,25,26] In chloroform ^1H and ^{13}C chemical shifts and coupling constants did not change significantly. Only the C-4–C-5 bond was affected with J_{4-5} = 4 Hz (11 Hz for cationomycin), which is interpreted as increased mobility of the rotor. Regardless of the direct effects due to the suppression of the aromatic ester function, the only significant differences recorded for the ^{13}C chemical shifts concerned C-25, -26 and -27 (2.3, −2.1, 3.7 ppm respectively) at the opposite end of the molecule. This observation confirms the globular conformation of the cationomycin skeleton. Though **1c** in $CDCl_3$ adopts a similar conformation to **1a**, this is not the case in methanol; the protic character of this solvent induces significant changes in the NMR parameters, thus J_{3-4} and J_{4-5} increases from 2.0 to 7.0 Hz and 4 to 6 Hz respectively. We explain these results by free rotation of the corresponding C-3–C-4 and C-4–C-5 rotors which is allowed in methanol, leading to the opening of the globular structure in the head-to-tail region; this may account for the observed changes in the ionophore and biological properties of **1c**.

Monensin. As for cationomycin, ^1H and ^{13}C chemical shifts in $CDCl_3$ for the salts with Li^+, Na^+, K^+, Rb^+, Cs^+ and NMe_4^+ are not very different in comparison with the free acid.[9] Complexation mainly affects the end bearing the carboxylic group, ring C and the 26-CH_2OH arm. For the chemical shifts, only 13% of the proton and 22% of the carbon resonances gave a correlation coefficient greater than 0.9 according to the ionic radius of the complexed cation. Only J_{7-8A} and $J_{25A-26B}$ coupling constants were affected. The small differences recorded for the NMR parameters between the alkaline complexes and the acid form of monensin suggest an identical globular conformation. The cation complexation process leads mainly to a rearrangement of the hydrogen bonds 11-OH → 1-O and 10-H → 2-O and a change in the positions of 9-O, 10-O and 11-O in the hemiacetal E. Due to the presence of a spiroacetal for A and B and of 18-Me for D in the envelope form, rings A, B and D are not very flexible and consequently little affected by the formation of the complex. Whatever the cation considered, differences recorded in the positions of 15-C, 14A-H and 14B-H resonances between the salts and the acid which corroborate the conformational modification of ring C. Interestingly, for the Na^+ complex specific variations were observed in comparison with the acid for J_{13-14A} (10.8–9.4 Hz) and J_{13-14B} (5.4–3.2 Hz), which indicates that the envelope conformation of ring C is converted into a half-chair by a pseudo-rotation in the complex. Coupling constants $J_{4-5,6-7,20-21,13-14A}$ and J_{13-14B} for Na^+ are also slightly different from the other cations, changing respectively from 13 to 11.5 Hz, 2.3 to 3.5 Hz, 4.1 to 2.5 Hz, 9.4 to 10.5 Hz and 3.2 to 5.5 Hz. These small variations correspond to local movements in the heterocycles, on overall better fitting of the cavity is accomplished for the sodium cation, given the well-documented monensin selectivity. This NMR study shows that the alkaline complexes of monensin all adopt a globular conformation similar to the free acid; the coordinating sites are 4-, 6-, 7-, 8-, 9- and 11-O, the complexing cavity being stabilised by hydrogen bonds 11-OH → 1-O and 10-OH → 2-O. These results are quite comparable to those obtained by X-ray diffractometry in the solid state.[27] One exception is for K^+ where ring B is clearly different in the solid state.[28] Differences in the dihedral angles between the acid and the 1:1 neutral complexes, for the backbone and rings do not exceed 15°, the main differences being recorded for rings C and E.

The carboxylate species (from the ammonium salt) is in a globular conformation close to the free acid in $CDCl_3$. The C-2–C-5 part remains identical in CD_3OD; this indicates that the anion conformation does not vary markedly in this solvent in contrast with what is observed for the acid. However, the

mobility of the rotor 20–21 is increased, J_{20-21} being modified from 2 to 4 Hz. It seems likely that the intramolecular chelations are retained in both solvents due to the carboxylate charge, as for the cationomycin anion.

Conclusions

This first systematic NMR study shows that in $CDCl_3$ solution cationomycin, monensin, their corresponding anion and 1:1 alkaline complexes adopt globular conformations with head-to-tail chelating interactions. These conformations are very similar in each series. Only local differences are observed between the acid and the other species for each ionophore, it is not possible to associate an ionic selectivity with a well-defined conformation. Further, on comparing the two bacterial ionophores the small differences observed cannot explain by themselves the pronounced selectivity in binding for sodium (monensin) and for potassium (cationomycin). The same observation can be made in CD_3OD except for the monensin free acid which presents a more open structure. On the contrary, very small conformational differences are recorded for cationomycin acid in $CDCl_3$ and CD_3OD; this rather unusual behaviour in the carboxylic polyether antibiotics group could be related to the presence of the aromatic ester moiety. Any relationship with the unusual biological properties observed for cationomycin and especially its low toxicity in mice still needs to be clarified.

Experimental

Chemicals.—Monensin was prepared by fermentation and purified in the laboratory. Cationomycin was obtained from Kaken Pharmaceutical (Japan) through a joint venture with Sanofi S.A. (France) and our laboratory. Monensin and cationomycin lithium and tetramethylammonium salts were prepared by exact neutralisation of the acid by the corresponding methoxide in MeOH. After evaporation of the methanol, the salt was dissolved in acetone and the solvent evaporated, this being repeated three times in order to remove last traces of water, and finally dried in a vacuum oven at 40 °C. Sodium and potassium salts were prepared by extraction of chloroform solution of acid ionophore by aqueous NaOH or KOH phase. The chloroformic phase was dried and evaporated. Rubidium and caesium salts were obtained by neutralisation of the corresponding hydroxide in methanol solution by an ionophore solution in the same solvent. The solvent was evaporated and the salt washed with ether. Deacylcationomycin was prepared according to ref. 3.

NMR Spectra.—The spectra were recorded on a Bruker MSL 300 instrument. The ROESY spectra of cationomycin and its potassium salt were also recorded on a Bruker WM 400. The operating frequencies were 300.13 or 400.13 MHz for ^1H and 75.47 MHz for ^{13}C. The concentrations of antibiotic used were about 5×10^{-2} mol dm^{-3}. No association took place as shown by the identical spectra in weak concentration (1×10^{-4} mol dm^{-3}). For ^1H spectra: SW = 3000 Hz with a digital resolution of 0.1 Hz/pt or 3×10^{-4} ppm/pt. and for ^{13}C spectra: SW = 15 000 Hz with a digital resolution of 1 Hz/pt or 1×10^{-2} ppm/pt.

COSY. The applied pulse sequence was ($\pi/2$, ^1H)–(t_1)–$\pi/4$ or $\pi/2$, ^1H)–FID, t_2). 512 experiments were recorded. Before Fourier transformation and symmetrisation, the data were multiplied with unshifted sine bell in each dimension.

COSY-DQF. The applied pulse sequence was ($\pi/2$, ^1H)–(t_1)–($\pi/2$, ^1H)–3 × 10^{-6} s–($\pi/2$, ^1H)–(FID, t_2) (using TPPI). 512 increments were recorded. Before Fourier transformation and phase-sensitive treatment, the data were multiplied with cosine bell squared in two dimensions.

^1H-^{13}C *Shift correlation.* The applied pulse sequence was ($\pi/2$, ^1H)–($t_1/2$)–(π, ^{13}C)–$t_1/2$)–(τ_1)–($\pi/2$, ^1H; $\pi/2$, ^{13}C)–(τ_2)–(BB, ^1H; FID, t_2)–(BB, ^1H; FID, t_2) with $\tau_1 = 0.00357$ s and $\tau_2 = 0.001785$ s. 256 or 512 experiments were recorded. Before Fourier transform, the data were multiplied (unshifted sine-bell in F2 and exponential in F1). The same procedure was used for ^1H–^{13}C long-range shift correlations except that τ_1 and τ_2 were adjusted for maximum polarisation for $J_{C-H} = 8$ Hz; $\tau_1 = 0.06$ s and $\tau_2 = 0.03$ s. HMBC sequence was also applied when attributions were difficult by classical ^1H–^{13}C long-range correlation.

ROESY. The applied sequence was ($\pi/2$, ^1H)–(t_1)–10^{-5} s–(spin lock, ^1H)–(FID, t_2) (^1H by decoupler). 512 experiments were recorded. Before Fourier transform and phase-sensitive treatment, the data were multiplied with cosine bell squared in each dimension.

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