ACETAL FORMATION IS RESPONSIBLE FOR THE NON-CLOSED FORM OF MONENSIN A IN METHANOLIC SOLUTION

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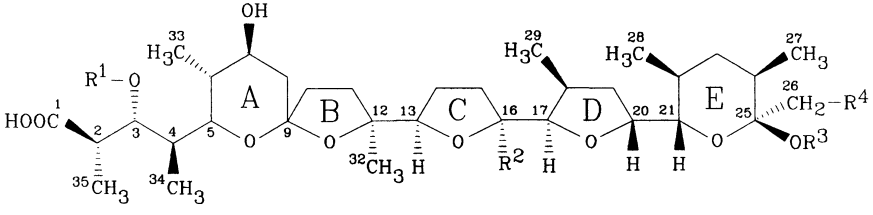
NMR evidence shows that so-called non-closed form of monensin A free acid reported to exist in methanol is in fact due to acetal formation at C-25. The 25-alkoxy derivatives retain this conformation even in non-polar media.

Key words: Polyether antibiotics; Ionophores; Monensin A; Methyl acetal formation; Conformational analysis; NMR spectroscopy, ^{1}H, ^{13}C.

Monensin A (**1**), a metabolite of *Streptomyces cinnamonensis*, was among the first polyether antibiotics discovered[1–3]. Nowadays its sodium salt is commercially available and widely used in veterinary medicine, especially in poultry industry against coccidiosis and for the improvement of food utilization in cattle production. Polyether antibiotics are natural ionophores facilitating transport of ions across membranes. Monensin itself exhibits a marked specificity to sodium ions.



1, $R^1 = CH_3$; $R^2 = CH_2CH_3$; $R^3 = H$; $R^4 = OH$
2, $R^1 = CH_3$; $R^2 = CH_3$; $R^3 = H$; $R^4 = OH$
3, $R^1 = CH_3$; $R^2 = CH_3$; $R^3 = CH_3$; $R^4 = H$
4, $R^1 = CH_3$; $R^2 = CH_2CH_3$; $R^3 = CH3$; $R^4 = OH$
5, $R^1 = CH_3CH_2CO$; $R^2 = CH_3$; $R^3 = H$; $R^4 = OH$
6, $R^1 = H$; $R^2 = CH_2CH_3$; $R^3 = H$; $R^4 = OH$
7, $R^1 = H$; $R^2 = CH_3$; $R^3 = H$; $R^4 = OH$

A special "closed" conformation (Fig. 1a), which is responsible for the cation binding, has been revealed by X-ray crystallography both for monensin free acid[4,5] and for several of its salts[6–10]. However a different

"open" conformation (Fig. 1b) was proposed for the acid form in methanol[11].

During our previous work in this field[12], we have isolated 25-*O*-methyl-26-deoxymonensin B (**3**) (monensin B (**2**) is a homologue of monensin A having methyl instead of ethyl at C-16) and noted that its conformation in chloroform resembled that of monensin free acid in methanol[11]. That led us to the preparation of 25-*O*-methyl monensin A (**4**) and a detailed spectroscopic study of this compound. We have used a simple reaction of monensin A free acid with methanol (see Experimental) instead of procedure described by Jeminet's group[13] (the treatment of sodium salt of monensin A with trimethyl formate in the presence of camphorsulfonic acid). In our hands, the reaction gave only one product, corresponding to the OMe_{ax} isomer (based on the comparison with published partial ^{13}C NMR data[13]). ^1H and ^{13}C NMR spectra were completely assigned (Tables I and II).

It is evident that the proton data, in particular vicinal couplings (for a comparison, see Table III) of **4** in both solvents are very similar to that of **1** in CD_3OD. Some scatter of chemical shifts and certain coupling constants between our results and those published by others[14–17] might be due to different temperatures of measurement, procedures used for data extraction, and a rather congested nature of the spectrum. The observed facts brought to mind the idea that the non-closed form of monensin free acid in methanol is in fact due to methyl acetal. An additional proof was obtained by repeating the Rodios and Anteunius experiment[11], *i.e.* by heating monensin A free acid in CD_3OD (40 °C, 12 h) and observing *both* ^1H and ^{13}C NMR spectra. ^1H NMR changes were identical to these described and ^{13}C NMR spec-

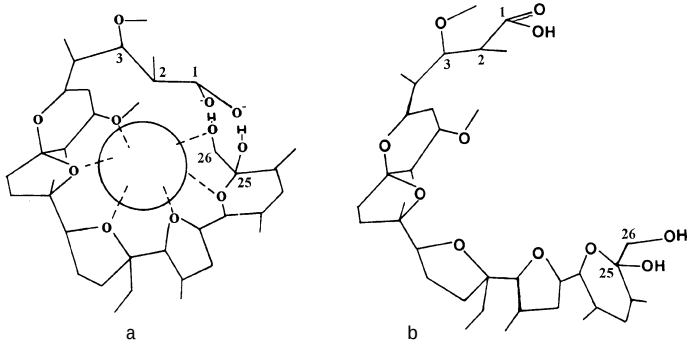


FIG. 1
Monensin A: a "closed" conformation, b "open" conformation

TABLE I
^1H and ^{13}C NMR data of 25-OMe monensin A (**4**) in $CDCl_3$

Position	δ_C, ppm	Multiplicity	δ_H, ppm	n_H	Multiplicity	*J*, Hz
1	178.96	s	–	0		
2	40.81	d	2.638	1	dq	5,1; 7.0
3	81.25	d	3.557	1	dd	5.1; 4.7
4	35.78	d	1.921	1	m	
5	67.98	d	4.035	1	dd	9.6; 2.3
6	36.90	d	2.079	1	ddq	3.0; 2.3; 6.9
7	71.47	d	3.774	1	ddd	3.2; 3.0; 2.7
8	31.84	t	1.658	1	ddd	14.1; 2.7; 0.9
			1.975	1	dd	14.1; 3.2
9	107.41	s	–	0		
10	39.14	t	1.867	1	m	
			1.944	1	m	
11	31.73	t	1.669	1	m	
			2.048	1	m	
12	86.29	s	–	0		
13	82.98	d	3.665	1	dd	9.1; 6.3
14	27.58	t	1.686	1	m	
			1.805	1	m	
15	34.61	t	1.714	1	m	
			1.978	1	m	
16	87.94	s	–	0		
17	85.58	d	3.933	1	d	4.4
18	33.50	d	2.296	1	m	
19	33.60	t	1.487	1	ddd	11.9; 7.3; 1.1
			2.255	1	ddd	11.9; 8.7; 7.0
20	76.96	d	4.292	1	ddd	8.8; 7.0; 3.3
21	77.52	d	3.474	1	dd	9.8; 3.3
22	32.75	d	1.363	1	m	
23	36.86	t	1.403	1	m	
			1.438	1	m	
24	34.76	d	1.974	1	m	
25	99.18	s	–	0		
26	63.05	t	3.531	1	d	11.3
			3.695	1	d	11.3
27	10.93	q	0.909	3	d	7.1
28	17.50	q	0.868	3	d	6.8
29	16.28	q	0.977	3	d	6.9
30	28.85	t	1.567	2	q	7.4
31	7.94	q	0.896	3	t	7.4
32	24.46	q	1.316	3	s	
33	11.94	q	1.015	3	d	6.9
34	15.65	q	0.868	3	d	6.8
35	12.33	q	1.224	3	d	7.0
36	58.12	q	3.366	3	s	
37	48.58	q	3.272	3	s	

TABLE II
^{1}H and ^{13}C NMR data of 25-OMe monensin A (**4**) in CD_3OD

Position	δ_C, ppm	Multiplicity	δ_H, ppm	n_H	Multiplicity	*J*, Hz
1	179.44	s	–	0		
2	42.66	d	2.611	1	dq	4.0; 7.0
3	83.66	d	3.661	1	dd	6.0 4.0
4	39.98	d	1.956	1	m	
5	69.48	d	4.125	1	dd	8.7; 2.3
6	38.77	d	2.033	1	m	
7	73.08	d	3.747	1	ddd	2.9; 2.4; 0.7
8	32.85	t	1.700	1	m	
			2.099	1	m	
9	108.97	s	–	0		
10	40.68	t	1.936	1	m	
			1.986	1	m	
11	32.56	t	1.585	1	m	
			2.148	1	m	
12	88.24	s	–	0		
13	84.30	d	3.744	1	dd	8.7; 6.3
14	29.36	t	1.710	1	m	
			1.891	1	m	
15	35.95	t	1.647	1	m	
			1.986	1	m	
16	89.71	s	–	0		
17	86.79	d	4.078	1	d	4.2
18	36.11	d	2.353	1	m	
19	34.89	t	1.533	1	m	
			2.394	1	m	
20	78.84	d	4.305	1	ddd	9.0; 6.8; 3.4
21	79.19	d	3.415	1	dd	9.8; 3.4
22	34.47	d	1.375	1	m	
23	38.73	t	1.306	1	m	
			1.414	1	m	
24	36.02	d	1.375	1	m	
25	101.14	s	–	0		
26	64.71	t	3.578	1	d	11.4
			3.630	1	d	11.4
27	11.81	q	0.962	3	d	7.1
28	18.39	q	0.904	3	d	6.0
29	17.00	q	0.994	3	d	6.8
30	30.72	t	1.616	2	m	
31	8.77	q	0.962	3	t	7.4
32	25.59	q	1.333	3	s	
33	13.22	q	1.022	3	d	6.9
34	16.62	q	0.917	3	d	6.8
35	12.41	q	1.190	3	d	7.0
36	59.19	q	3.388	3	s	
37	50.15	q	3.271	3	s	

TABLE III
Comparison of proton–proton coupling constants J (in Hz) in compounds **1**, **1a**, and **4**

J_{ij} [a]	**1**[c] $CDCl_3$	**1a**[b,c] $CDCl_3$	**4** CDC_{l3}	**1**[c,f] CD_3OD	**1a**[c] CD_3OD	**1**[d] CD_3OD	**4** CD_3OD
2, 3	10.2	10.2	5.1	4.0	10.2	5.0	4.0
2, 36	6.7	6.7	7.0	7.0	6.8	7.0	7.0
3, 4	2.0	1.6	4.7	5.4	1.6	5.6	6.0
4, 5	11.5	13.0	9.6	8.6	11.3	8.5	8.7
4, 34	6.8	6.9	6.8	7.0	6.8	7.0	6.8
5, 6	1.9	1.8	2.3	2.4	2.0	2.0	2.3
6, 7	>2.0	2.3	3.0	3.0	n.d.[g]	4.3	2.9
6, 33	7.1	6.9	6.9	7.2	7.2	7.2	6.9
7, 8 d	3.5	3.6	3.2	3.2	n.d.	2.9	2.4
7, 8 u	>2.0	2.0	2.7	2.8	n.d.	5.6	0.7
8 d, 8 u	14.5	14.1	14.1	14.0	n.d.	15.0	n.d.
13, 14 d	16.0[e]	15.3[e]	6.3	14.6[e]	4.8	5.6	6.3
13, 14 u	16.0[e]	15.3[e]	9.1	14.6[e]	10.2	8.6	8.7
17, 18	4.0	3.4	4.4	4.2	3.3	4.5	4.2
18, 29	6.8	7.1	6.9	7.1	>6.2	7.0	6.8
19 d, 20	5.8	7.0	7.0	6.8	20.6[e]	7.0	6.8
19 u, 20	10.5	9.8	8.7	9.8	20.6[e]	8.5	9.0
20, 21	2.6	4.1	3.3	3.4	3.7	4.5	3.4
21, 22	10.6	9.5	9.8	10.0	9.7	10.0	9.8
22, 28	6.6	5.9	6.8	6.7	5.7	5.8	6.0
24, 27	6.3	5.6	7.1	6.6	6.4	6.8	7.1
26 d, 26 u	11.3	11.9	11.3	11.4	11.8	11.5	11.4
30, 31	7.4	7.6	7.4	7.5	7.0	8.6	7.4

[a] u Upfield, d downfield; [b] **1a** is sodium salt of monensin A; [c] ref.[11]; [d] ref.[16]; [e] ΣJ; [f] "aged" solution values (48 h); [g] not determined.

trum of the final product (almost pure single compound) was identical to that of **4** within experimental error with one exception – a missing signal of C-37, replaced by a CD_3 group (high value of similarity index[18] S = 0.9958 serves as a proof of identity). Therefore, the non-closed form of monensin (acid) in methanol belongs to its perdeuteriomethyl acetal. The reason for similar behaviour of nigericin[11] is probably the same.

Interesting conclusions could be reached by examination of several structure–activity relationships observed in this family. Laidlomycin (**5**) (formally derived from monensin B and containing a propionyloxy instead of methoxy group at C-3) has biological activity similar to monensin[19,20]. On the other side, both 3-*O*-demethylmonensins A and B (**6** and **7**) are inactive[21]. Monoacylation at C-26 of laidlomycin produces compounds with improved properties[22]. Both natural and semisynthetic urethanes of monensin[23] and laidlomycin[24] possess an increased ion-transporting capability, decreased specificity towards sodium, and some of them are even divalent ionophores. The removal of both C-25 substituents leading to the corresponding lactone, the formation of C-25, C-26 acetonide, and alkylation of the C-25 hydroxyl[13,18] result in poor complexing properties and the absence of any biological activity. All the above summarized facts stress the importance of the free C-25 hydroxyl for the formation of "closed" conformation of monensin (and its analogues) necessary for proper ion complexation. The "open" conformation is restricted to derivatives modified in the ring E, and it is an inherent property of these structures persisting also in non-polar solvents. A computational study of monensin in gaseous state[17] did not provide a good candidate for the "open" conformation (Fig. 1b) and the authors concluded that a molecular dynamics modelling in the solvent is clearly needed.

EXPERIMENTAL

NMR spectra were measured on Varian VXR-400 and INOVA-400 spectrometers (observing frequency for ^1H: 399.95 and 399.91 MHz, for ^{13}C: 100.58 and 100.57 MHz, respectively) in $CDCl_3$ or CD_3OD at 30 °C. Multiplicity of carbon signals was determined by APT and DEPT experiments. The reported assignment is based on 2D NMR experiments (HOM2DJ, COSY, delay-COSY, RELAY, TOCSY, HETCOR and HMQC) performed using the manufacturer's software. Coupling constants given in Tables I and II were extracted using the first-order approximation. Both positive- and negative-ion FAB spectra were measured on a Finnigan MAT 95 double focussing instrument equipped with a standard saddle field FAB gun (IonTech, Teddington, U.K.) operated at 2 mA current and 6 keV energy, using xenon as a bombarding gas ($1 \cdot 10^{-5}$ mBar) and 3-nitrobenzyl alcohol as a matrix.

Mixture of sodium salts of monensins A and B was isolated from the culture of *Streptomyces cinnamonensis*, exctracted and separated as described previously[25].

Preparation of 25-*O*-Methyl Monensin A (**4**)

The sodium salt of monensin A (20 mg) was dissolved in $CHCl_3$ (5 ml, ethanol present as a stabilizer was removed by passing through a short silica gel column) and three times washed with 1 M HCl (10 ml). Water phase present in chloroform solution of monensin A free acid was removed by repeated centrifugation. The solvent was evaporated and the solid residue was dissolved in methanol (20 ml). Reaction was carried out at 30 °C and checked[21] by TLC until the monensin A free acid disappeared. The conversion to **4** (an amorphous solid, positive-ion FAB *m/z* 707.5 $[M + H + NA]^+$, negative-ion FAB *m/z* 683.5 $[M - H]^-$) was nearly quantitative.

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