The Conformation of 6-Methoxyerythromycin A in Water Determined by Proton NMR Spectroscopy

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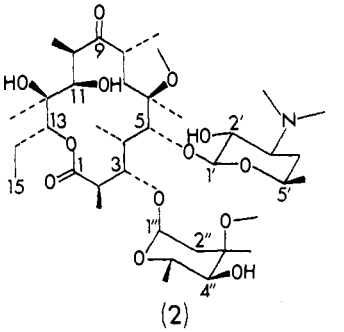
Received September 8, 1992

The proton NMR spectrum of an aqueous solution of 6-methoxyerythromycin A (2) has been assigned and nuclear Overhauser effects have been obtained from a series of NOESY spectra. Carbon-13 antisymmetric spin-lattice relaxation times have been measured for the methylene and methine carbons. The NMR data show that 2 is present exclusively in the keto form and has a conformation very similar to that reported for erythromycin A (1) in the solid and solution phases.

Introduction

Erythromycin A (1) is a macrolide antibiotic which is particularly useful in treating infections by Gram-positive bacteria.[1] Consequently 1 and its derivatives have been the focus of a large number of structural and conformational studies. The structural literature on crystalline erythromycin and its solutions in organic solvents have shown that the conformation of 1 in the solid state[2] is very similar to that in chloroform solution.[3] Although the activity of 1 is expressed in an aqueous environment, no study of its conformation in water has been reported and the parameters of its proton NMR spectrum in water have not been published. This NMR study was initiated to fill the gap in the literature.

The covalent structure of 1 consists of a 14-membered erythronolide B ring with α-L-cladinose and β-D-desosamine sugar substituents in the 3 and 5 positions, respectively. NMR measurements have shown the main, 9-keto form is in equilibrium with a cyclic hemiketal.[4,5] We selected the derivative 6-methoxyerythromycin (2) (Figure 1) for NMR measurements in water with the expectation that methylation at the 6 position would block ketal formation. The NMR data confirmed this expectation. We only observed a single isomer, the 9-keto form, and determined its conformation in water.



(2)

Figure 1. Covalent structure of 2.

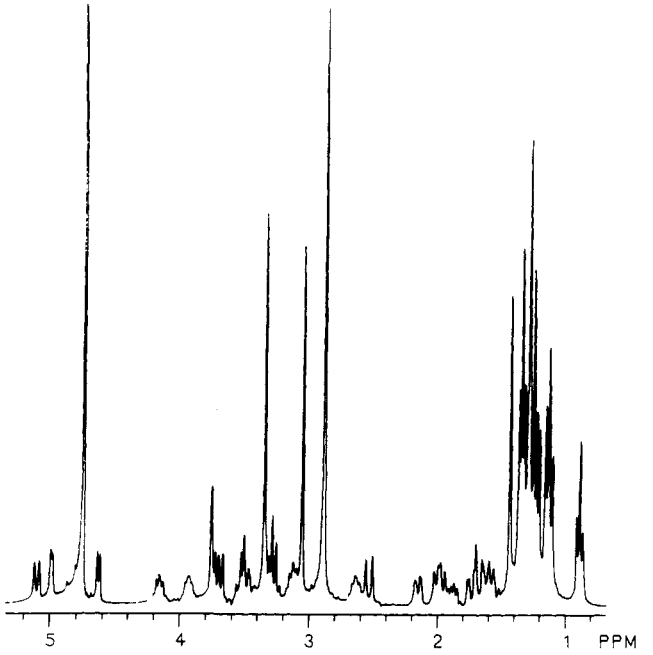


Figure 2. 300-MHz proton NMR spectrum of a 30 mM aqueous solution of 2 at pD 5.9 and 30.0 °C.

Results and Discussion

I. Assignment of the NMR Spectrum. The proton spectrum of a 30 mM aqueous solution of 2 (Figure 2) which has many well-resolved features was assigned to a single isomer. The carbon-13 spectrum with carbonyl resonances at 224.5 and 178.1 ppm confirms that this isomer is the 9-keto form. A complete assignment of the proton spectrum tabulated in Table I was achieved with the information from phase-sensitive COSY, relay-COSY, and NOESY spectra. The parameters in water are very similar to those observed in $CDCl_3$.[6]

The NMR results show that methylation at the 6 position was effective in blocking the formation of a cyclic 9-6

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0022-2623/92/1835-4842$03.00/0

Table I. Proton Chemical Shifts and Coupling Constants for 1 and 2

	2 in D_2O at 30.0 °C[a]		1 in $CDCl_3$ at 21–23 °C[b]	
carbon	δ (ppm)	^3J (Hz)	δ (ppm)	^3J (Hz)
2	3.11	9.8 (2, 3)	2.87	9.5 (2, 3)
2-Me	1.28	8.6 (2, 2-Me)	1.12	7.1 (2, 2-Me)
3	3.66	<1.5 (3, 4)	3.99	1.4 (3, 4)
4	1.97	7.8 (4, 5)	1.97	7.8 (4, 5)
4-Me	1.10	7.8 (4, 4-Me)	1.10	7.5 (4, 4-Me)
5	3.73		3.56	
6-Me	1.42		1.46	
6-OMe	3.04			
7a	1.98	10.0 (7a, 8)	1.93	11.9 (7a, 8)
7e	1.67	1.7 (7e, 8)	1.74	2.4 (7e, 8)
8	2.67		2.68	
8-Me	1.20	7.0 (8, 8-Me)	1.16	7.0 (8, 8-Me)
10	3.30	<1.5 (10, 11)	3.08	1.4 (10, 11)
10-Me	1.13	7.0 (10, 10-Me)	1.14	6.9 (10, 10-Me)
11	3.75		3.82	
12-Me	1.24		1.12	
13	5.09	11.0 (13, 14a)	5.03	11.0 (13, 14a)
		2.0 (13, 14e)		2.3 (13, 14e)
14a	1.60	7.4 (14a, 15)	1.48	7.4 (14a, 15)
14e	1.88	7.4 (14e, 15)	1.91	7.4 (14e, 15)
15	0.87		0.84	
1′	4.62	7.0 (1′, 2′)	4.40	7.2 (1′, 2′)
2′	3.51	ca. 9 (2′, 3′)	3.21	10.3 (2′, 3′)
3′	3.51	12 (3′, 4a′)	2.43	12.3 (3′, 4a′)
3′-NMe	2.87		2.29	
4a′	1.56	10 (4a′, 5)	1.22	10.8 (4a′, 5′)
4e′	2.14	2 (4e′, 5′)	1.67	2.1 (4e′, 5′)
5′	3.92		3.48	
5′-Me	1.32	6.2 (5′, 5′-Me)	1.22	6.1 (5′, 5′-Me)
1″	4.98	4.6 (1″, 2″a)	4.88	5 (1″, 2″)
2a″	1.72		1.56	
2e″	2.52		2.35	
3″-Me	1.27		1.23	
3″-OMe	3.34		3.31	
4″	3.26	9.3 (4″, 5″)	3.00	9.7 (4″, 5″)
5″	4.14		3.99	
5″-Me	1.34	6.2 (5″, 5″-Me)	1.27	6.2 (5″, 5″-Me)

[a] This work. [b] Reference 6.

hemiketal. This result is strongly suggested by the crystal structure of 1 in which the oxygen on carbon 6 is within 3 Å of carbon 9 and is poised to form a stable five-membered ring.[2] We are puzzled by the claim of Barber et al. that the hemiketal is the result of 9–12 rather than 9–6 cyclization as the oxygen on carbon 12 is more than 4 Å from carbon 9 in the crystal structure and a significant change in the conformation of the macrolide ring would be required to reduce the distance.

II. Conformation of 2. Before discussing the results of 2, it is worth reviewing the most prominent features of the crystal structure of 1 which Everett and Tyler found was an excellent model for the dominant conformer of 1 in chloroform.[2,3] Both sugar rings adopt a chair conformation and are oriented approximately perpendicular to the macrolide ring with the 6′-Me and 6″-Me substituents pointing up. Everett and Tyler refer to this as the "up–up" conformation. With this arrangement the two rings face each other. The macrolide ring adopts a conformation very close to the Perun conformation, a modification of an alternate diamond-lattice conformation.[7,8] In this conformation designated by Everett and Tyler as "folded-out" the 11-hydrogen is close to the 4-hydrogen but not the 3-hydrogen and the 3- and 5-hydroxyls in the aglycon bear a cis periplanar relationship to one another.

The principal NMR determinants of the conformation of a molecule are coupling constants, which depend on dihedral angle and nuclear Overhauser effects (NOE), which depend on proton–proton distances. The set of coupling constants (Table I) and the pattern of NOE's (Table II) for 2 in water very closely match those reported by Everett and Tyler for 1 in $CDCl_3$.[3] Additional NOE's for 2 are due to the additional methyl group, but these are features which could be extrapolated from the crystal structure. For example, the proximity of the 6-hydroxyl to the 11-hydrogen in the crystal structure of 1 corresponds to the NOE across the ring between the 6-methoxy and the 11-hydrogen in 2. The set of chemical shifts are slightly different and the values in water are close enough for some protons that the NOESY cross peak cannot be resolved from the diagonal. This technical difficulty accounts for the missing NOE's. Given the preponderance of the evidence, these details in no way affect the principal conclusion. Solid 1 and the dominant conformer of 1 in chloroform and 2 in water have the same or very similar conformation.

III. Conformational Equilibria. The chair conformation of the two sugar rings and their relative orientation are particularly well-established for 2, 1, and other derivatives of 1 studied by Everett and Tyler.[9,10] There is no indication of multiple forms involving these aspects of the conformation. In contrast, temperature and solvent effects on the coupling constants $^3J_{2,3}$, $^3J_{7e,8}$, and $^3J_{7a,8}$ have been presented as evidence for an equilibrium between major and minor conformers of 1.[3,8] An NOE between the 3- and 11-hydrogens has been attributed to the minor conformer.[3] The other coupling constants are invariant within experimental error and the interconversion of the conformers was presumed to involve global rather than local changes. Small variations in NT_1 of the methine and methylene carbons of 1 in $CDCl_3$ indicated that reorientation of the molecule is fairly isotropic and that the molecule does not undergo large-amplitude vibrations.[3,11]

The data for 2 indicate that its global conformation is well-defined. T_1[a], the antisymmetric spin–lattice relaxation time, was measured for the methylene and methine carbons using an INEPT polarization transfer method.[12] The values of NT_1[a] at 30.0 °C did not differ from the average, 0.17 ± 0.013 s, by more than the 95% confidence interval, so the molecule is fairly rigid. The proton spectrum was measured over the range 19–50 °C and statistically significant variations of the coupling constants were not observed. Small variations in δ_7, and δ_{10Me}, 0.06 and 0.03 ppm over the 31 °C range, indicate conformation

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Table II. NOE's for **2** and **1**[a]

	2	2-M	3	4	4-M	5	6-M	6-O	7a	7e	8	8-M	10	10-M	11	12-M	13	14a	14e	15	1′	2′	3′	4′a	4′e	5′	5′-M	1″	2″a	2″e	3″-M	3″-O	4″	5″	5″-M
2		W	M	M	M																														
2-M	m		W																									W		W					
3	w	w		M				M																				M							
4	m		m		W										M																				
4-M	m			m		W															M														
5			m		w		M	W													S													S	
6-M						s		W		W	M																								
6-O															M																				
7a										S			W		W																				
7e							w		m		W	W																							
8							s		w	w		M																							
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11			w	m					w				m			M	W																		
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2″a																												w		S	W		W		
2″e																												w	m		W	M			
3″-M																							w						w	w		W	W		
3″-O					w																w									m	w				
4″																													w		w			W	M
5″						m															m					w									S
5″-M																										w							s	m	

[a] The data above the diagonal are for **2**, where S, M, and W indicate upper bounds on the interproton distances: S, <2.2 Å; M, <2.5 Å; W, <3.0 Å. The data below the diagonal are for **1** (ref 3), where s, m, and w indicate >5%, 2–5%, and 0–2% NOE effects. M = methyl and O = methoxy.

averaging, but the magnitude of the effect suggests that the concentration of minor species is small. It was not possible to check for an NOE between the 3- and 11-protons as the NOESY cross peak would be too close to the diagonal to be resolved.

Conclusions

The proton NMR spectrum of 6-methoxyerythromycin A, 2, in water has been assigned to a single species with the aid two-dimensional techniques. Small temperature variations of the chemical shifts indicate that although more than one conformer is present in water, one conformer dominates. The set of proton–proton coupling constants and nuclear Overhauser effects yield a structure for the predominant conformer that matches that reported for erythromycin A, 1, in the solid state and in chloroform. The invariance in conformation indicates that 2 will not change conformation when it goes from an aqueous to a hydrophobic environment.

Experimental Section

A sample of 2, which was shown to be better than 98% pure by thin-layer chromatography and HPLC, was provided by Abbott Laboratories. A saturated solution of 2 was prepared in D_2O and the pD was adjusted to 5.9 with the addition of dilute DCl. The solution was lyophilized and redissolved in 0.5 mL of 99.8% D_2O (Aldrich). The final concentration of the sample was 30 mM. A final step was performed on a vacuum line to prepare the sample used in the measurement of NOE's. The solution was lyophilized in a 5-mm NMR tube, 0.5 mL of 99.96% D_2O (Aldrich) was distilled to the NMR tube, and the tube was sealed off.

Proton NMR measurements were made at 300.15 MHz and carbon-13 measurements at 75.48 MHz on a General Electric QE-300 NMR spectrometer. The water peak was used as an internal standard for the proton chemical shifts. The chemical shift of the water relative to TSP was determined in a separate experiment. TPPI COSY and TPPI Relay-COSY spectra were collected at 25.0 and 30.0 °C with 2048 points in the t_2 direction and 300 values of t_1. The free-induction decay was zero-filled once in both dimensions. Phase-sensitive NOESY spectra were acquired at 22.0 and 30.0 °C with 4096 points in the t_2 direction and 256 values of t_1. The free induction decay was zero-filled once in the t_1 dimension. A spectrum with a mixing time of 0.500 s was used for assignment purposes. The rate of growth of the NOESY cross peaks was obtained at 30.0 °C from a series of three consecutively measured spectra with mixing times of 0.100, 0.200, and 0.400 s. The volumes of the cross peaks were measured, and the slope of the linear portion of cross-peak volume versus time was determined. Distances were obtained by comparing each slope to that for methylene protons and by assuming that the slopes were inversely proportional to the sixth power of the proton–proton distance. This approach has been shown to yield reliable upper bounds for distances.[13] A standard inversion recovery sequence with a composite 180° pulse was used to measure ^1H T_1's at 25.0 °C. A relaxation delay of 5.0 s and a 32K data block were used.

A normal decoupled carbon-13 spectrum and an APT spectrum were measured at 25.0 °C using Waltz decoupling, a 1.8-s relaxation delay, a 64K block size, and a 30° pulse angle. The low concentration of the sample required 100 000 acquisitions and use of exponential multiplication to reduce noise and double precision for storage of the free-induction decay. Carbon-13 relaxation times were acquired at 25.0 °C using an INEPT preparation period developed by Marion et al. to enhance the signal-to-noise ratio.[12] The pulse sequence yields the relaxation time T_1^e rather than T_1. In a series of five runs the delay between the last proton pulse and the last carbon-13 pulse was varied between 10 μs and 500 ms. The following changes were made in the parameters: block size, 16K; relaxation delay, 2.3 s.

Acknowledgment. We would like to thank Abbott Laboratories for a generous donation of 6-methoxyerythromycin. R.B. and W.E.S. received a Partners in Science grant from Research Corporation. J.T. and D.P., undergraduate research participants, were supported by the Robbins Fund at Pomona College. Initial carbon-13 measurements were performed by Sean Keele.

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