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Conformational Analysis of Azithromycin by Nuclear Magnetic Resonance Spectroscopy and Molecular Modelling

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Abstract *The conformation of azithromycin 1 in the solution was determined by NMR spectroscopy and molecular mechanics calculations and compared with its crystal structure and with some erythromycin derivatives In solution 1 exists predominantly in a "folded-in" conformation in the C-3 to C-5 region, whereas its crystal state conformation is "folded-out"*

Azithromycin **1** is the first member of a new class of antibiotics called azalides[1,2] It is an effective therapeutic agent for oral treatment of sexually transmitted diseases, upper and lower respiratory tract infections, and skin structure infections[3] Azithromycin differs structurally from erythromycin A **2** by insertion of a methyl-substituted nitrogen at position 9a in the lactone ring to create a 15-membered macrocycle This modification produces an enhanced spectrum and potency against bacteria, superior stability in an acid environment, as well much longer elimination half-lives and much higher tissue concentrations compared with erythromycin A **2**[4]

The crystal structure of **1** is known from an X-ray crystallographic analysis of its dihydrate[2] (Fig 1a) In view of the aforesaid interesting biological properties, it seems worthwhile to investigate the solution-state

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conformation behavior of **1** and compare to that of **2**[5] Since such analyses are based largely on NMR spectroscopy, the NMR characterization of **1** was prerequisite for these studies The assignments of the NMR spectra of **1** have been determined by a combination of 2D NMR methods[6,7]

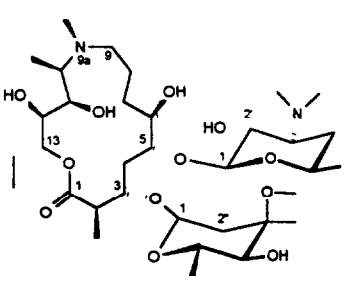
The conformation study presented here had four main aims

(i) the determination of the solution conformation of azithromycin **1** by analyzing ^1H ^3J coupling constants and molecular mechanics calculations,

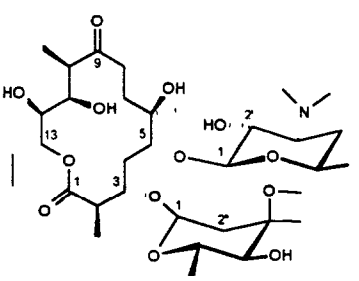
(ii) to obtain information concerning the spatial proximity of sugar and macrocycle moieties using 2D NOESY experiments,

(iii) the determination of the motional properties of the methyl groups,

(iv) the comparation of the solution conformation of **1** with its crystalline-state conformation and conformation of erythromycin A **2** and dirithromycin **3**



1



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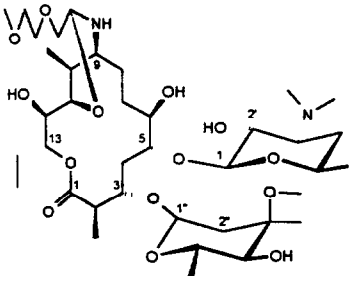

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Solvent effect

Small variations in the chemical shifts (≤ 0 24 ppm) are observed between $CDCl_3$ and CD_3OD (Table 1) In the macrocycle, these changes are slightly enhanced for the protons situated close to the nitrogen and lactone (7_{ax}, 9_{ax}, 10, 11, 13) In the sugar ring there is a particular effect for the protons located on the two faces of

Table 1 ^1H NMR Chemical Shifts for Azithromycin **1** in $CDCl_3$ and CD_3OD and diastereotopic ^1H Chemical Shift Differences ($\Delta\delta$) in ppm

H	$CDCl_3$ 293 K		CD_3OD 293 K		H	$CDCl_3$ 293 K		CD_3OD 293 K		H	$CDCl_3$ 293 K		CD_3OD 293 K	
2	2 73		2 79		14eq	1 89	(0 43)	1 87	(0 39)	4'eq	1 67	(0 78)	1 73	(0 54)
3	4 29		4 25		14ax	1 46		1 48		4'ax	1 23		1 19	
4	1 99		2 01							5'	3 51		3 75	
5	3 64		3 69		14Me	0 89		0 89		5'Me	1 24		1 18	
7eq	1 80	(0 55)	1 77	(0 41)	2Me	1 20		1 21		3'NMe_2	2 29		2 33	
7ax	1 25		1 36		4Me	1 05		1 05						
8	2 02		2 00		6Me	1 32		1 32		1"	5 19		5 05	
9eq	2 55	(0 50)	2 53	(0 40)	8Me	0 91		0 92		2"eq	2 37	(0 43)	2 43	(0 54)
9ax	2 05		2 13		10Me	1 09		1 09		2"ax	1 59		1 58	
10	2 69		2 77		12Me	1 10		1 10		4"	3 04		3 04	
11	3 69		3 61		9aNMe	2 32		2 29		4"OH	2 16		2 16	
11OH	5 19		-							5"	4 09		4 20	
12OH	3 04		-		1'	4 44		4 53		5"Me	1 34		1 28	
13	4 70		4 83		2'	3 24		3 25		3"Me	1 25		1 25	
					3'	2 44		2 68		3"OMe	3 35		3 37	

Table 2 ^3J Coupling Constants (in Hz) for **1** in Various Solvents

^3J		Solvent					
		$CDCl_3$	$CDCl_3$	$[^2H_5]Py$	$(CD_3)_2CO$	CD_3OD	$[^2H_6]DMSO$
		293 K	318 K	293 K	293 K	293 K	293 K
2	3	3 6	4 5	4 4	4 6	4 7	4 7
3	4	1 7	2 2	2 2	2 2	2 2	1 9
4	5	7 4	7 6	7 4	7 2	7 6	7 4
7ax	8	≈11	11 1	b	11 7	10 7	b
7eq	8	1 5	1 5	a	b	1 0	a
8	9ax	≈10	≈10	b	b	10 7	9 5
8	9eq	1 2	a	a	2 8	≈1	1 5
10	11	1 9	1 6	a	b	≈1	a
13	14ax	9 9	9 6	9 8	9 9	9 6	9 9
13	14eq	2 5	2 8	2 5	2 6	2 8	2 6
1'	2'	7 3	7 5	7 7	7 2	7 7	7 5
2'	3'	10 2	10 4	9 8	9 1	10 4	9 9
3'	4'ax	10 9	10 7	11 8	10 3	10 8	10 9
3'	4'eq	3 4	3 8	3 9	3 6	3 8	3 6
4'ax	5'	10 9	10 4	10 3	b	10 7	10 3
4'eq	5'	2 2	1 8	1 3	b	1 9	1 6
1"	2"ax	4 8	4 9	5 0	4 6	5 5	4 8
1"	2"eq	a	1 5	a	a	a	a
4"	5"	9 4	9 3	9 1	9 2	9 7	9 6

[a] Not resolved
[b] Overlapped

desosamine (5', 4_{eq}, 3', 1') and cladinose (5", $2"_{eq}$) Moreover, these protons are concerned in the inter-sugar interactions observed by NOE experiments (see Nuclear Overhauser Enhancement Experiments) This suggests that these two regions of the sugars are face to face

Conformational analysis of 1 in the solution state

An analysis of the ^1H NMR ^3J values was used to compare the major solution-state conformation of azithromycin and that in the crystalline state

The structure of this compound obtained by X-ray analysis showed that the erythronolide and the sugar rings have the same conformation as in erythromycin A, except of course, in the region of the ring-enlargement (9a N-methyl)[2] Both sugar components had chair conformation with the maximum number of substituents in the more stable equatorial positions The sugar rings are oriented nearly perpendicularly to the macrocyclic lactone ring

From the observed ^1H coupling constant (Table 2) it is found that the sugar rings adopt in solutions the same chair conformations as observed in the crystal structure, with similar dihedral angles (Table 3) The vicinal coupling constants for the X-ray structure were calculated by using a modified Karplus equation[8]

$$^3J_{HH}=P_1*\cos^2\phi+P_2*\cos\phi+\Sigma\Delta\chi_i\{P_4+P_5*\cos^2(\xi_i*\phi+P_6*|\Delta\chi_j|\} \quad (1)$$

(see Experimental) and the values obtained for the two sugar rings agree well with those measured experimentally (Table 3) The large values of the axial couplings $^3J_{2',3'}$, $^3J_{3',4'ax}$, $^3J_{4'ax,5'}$ and $^3J_{4",5"}$ (9 4-10 9 Hz) indicated little or no population of the ring-inverted chair conformations of the two rings

For the lactone ring we first examined the trans coupling constants with large $^3J_{HH}$ values $^3J_{8,9}$=10 7 Hz, $^3J_{13,14}$=9 9 Hz These values are in good agreement with the calculated couplings for the crystalline state conformation $^3J_{8,9}$=11 6, $^3J_{13,14}$=11 1 Hz The differences, which are almost within experimental error, can be interpreted as a slight variation of the dihedral angles, H8-C8-C9-H9 and H13-C13-C14-H14

The difference for $^3J_{4,5}$ between the experimental value (7 4 Hz) and the calculated for X-ray conformation (6 2 Hz) may have been due to the steric effect In the eclipsed Newman projection ($\phi_{X\text{-}ray}$=130 2°) the steric effect of the sugar ring on C5 and the methyl on C4 is preponderant and the resulting torsion angle, H4-C4-C5-H5, could be slightly disturbed in solution A different orientation of these substituents could increase ϕ by ca 7° The very low values for the lesser three-bond H,H coupling constants (Table 2) indicate that the region corresponding to these bonds $^3J_{8,9}$, $^3J_{10,11}$, $^3J_{13\ 14}$ remains in a similar conformation in solution to that in the solid state

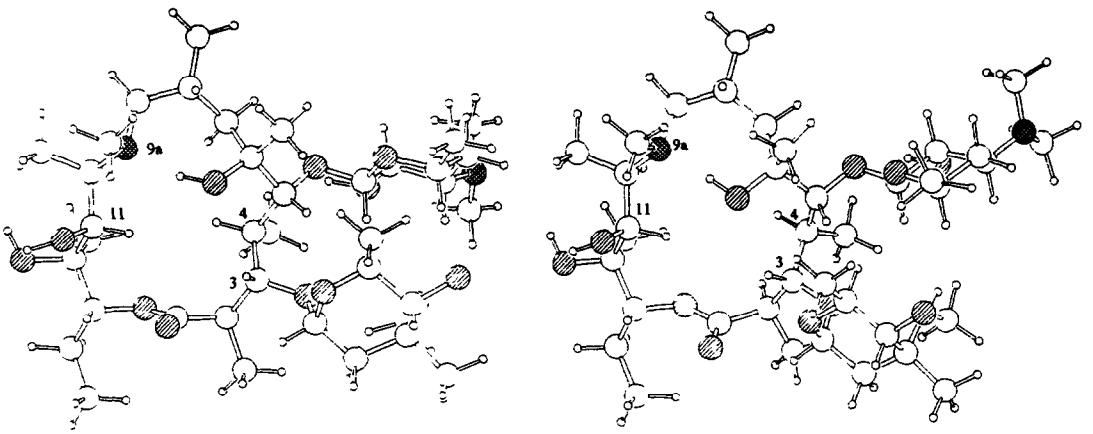
The differences for ^3J with respect to the calculated values are more important for $^3J_{2\ 3}$ and $^3J_{7,8}$ The experimental $^3J_{2,3}$ value is 3 6 Hz ($CDCl_3$) while the calculated coupling constant for the solid state conformation is 9 7 Hz These values are in agreement with two types of conformations of erythromycin derivatives the C3 to C5 "folded-in" ($^3J_{2,3}$=3 6 Hz) and the C3 to C5 "folded out" type ($^3J_{2,3}$=9 7 Hz) respectively The "folded-in" conformations B and B' are based on the crystal structures of dirithromycin[9] (B) and 11-ether derivative of 9-methoxyiminoerythromycin[10] (B'), respectively, whilst the "folded-out"

Table 3 Coupling Constants for Vicinal Proton Pairs and Corresponding Dihedral Angles for **1**

Vicinal proton pair		$^3J^a_{exp}$/Hz	ϕ^b_{calc}/°	$\phi_{X\text{-}ray}$/°	$^3J^c_{X\text{-}ray}$ Hz	ϕ^d_{MM2}/°	$^3J^e_{MM2}$/Hz
H-2,	H-3	3 6	116	151 8	9 7	110 4	2 7
H-3,	H-4	1 7	-60	-64 7	1 3	-73 8	0 7
H-4,	H-5	7 4	137	130 2	6 2	142 7	8 5
H-7_{eq},	H-8	1 5 [f]	-75	-110 0	2 5	-86 4	1 0
H-7_{ax},	H-8	11 1 [f]	161	134 8	6 7	161 0	11 1
H-8,	H-9_{eq}	1 2	79	68 6	2 5	64 3	3 0
H-8,	H-9_{ax}	10 7 [g]	-164	-174 9	11 6	178 2	11 8
H-10,	H-11	1 9	59	82 1	0 6	74 6	0 8
H-13,	H-14_{eq}	2 5	57	70 9	1 2	66 3	1 6
H-13,	H-14_{ax}	9 9	-162	-172 9	11 1	-175 5	11 3
H-1',	H-2'	7 3	171	174 3	7 5	172 2	7 4
H-2',	H-3'	10 2	-176	-172 1	9 9	178 0	10 5
H-3',	H-$4'_{eq}$	3 4	61	61 2	3 5	56 6	4 1
H-3',	H-$4'_{ax}$	10 9	164	177 3	11 7	175 1	11 7
H-$4'_{eq}$,	H-5'	2 2	-60	-59 2	2 3	-58 4	2 3
H-$4'_{ax}$,	H-5'	10 9	-157	-174 6	11 7	-176 0	11 6
H-1",	H-$2"_{eq}$	1 5 [f]	77	70 6	1 9	65 3	2 3
H-1",	H-$2"_{ax}$	4 8	-44	-43 1	4 9	-49 2	4 1
H-4",	H-5"	9 4	180	174 3	9 2	175 5	9 3

[a] Experimental values for 1 in $CDCl_3$ at 293 K
[b] Dihedral angles ϕ_{calc} were calculated from $^3J_{exp}$ using equation (1) by simple Turbo Pascal program
[c] Coupling constants calculated for X-ray geometry using equation (1)
[d] Dihedral angles of solute state conformation predicted on base MM2 calculation
[e] Coupling constants calculated for MM2 solution state geometry using equation (1)
[f] $CDCl_3$, 318 K
[g] CD_3OD, 293 K

Fig 1 Ball-and-stick representation of the crystal (a) and solution state (b) structure of azithromycin 1



conformation (A) is based on the crystal structure of erythromycin A hydroiodide dihydrate[11] In the "folded-in" conformations B and B' rotation about C2 to C3 bond causes the inward folding of the C3 to C5 portion of the lactone ring so greatly eases the steric hindrance on 2Me, but forces 4Me into a more hindered environment B and B' type differ from each other mainly in the C6 to C9 region[10] The "folded-out" conformation is characterized by a close cross-ring approach of H4 and H11 and the rotation of 2Me is subject to much greater steric hindrance than that of 4Me However the similarity of $\phi_{H3,H4}$ and $\phi_{H4,H5}$ in the crystal and solution state conformations means that the orientations of the sugar rings with respect to one another remains the same, even though in solutions they are now "folded-in" towards the C9-C12 side of the ring

The observed values for $^3J_{7eq,8}$=1 5 Hz and $J_{7ax,8}$=11 1 Hz, respectively, are similar to those of erythromycin A[5], and correspond to the dihedral angles in trans diequatorial and trans diaxial directions, respectively In the crystal structure of **1**, however, C7-C8 region is in the partially eclipsed conformation

In order to obtain the precise geometry of the solution state conformation of **1** we have performed molecular mechanic calculation by MM2 program[12] The crystal state geometry of **1**[2] was modified in regions of main differences for experimental $^3J_{HH}$ with respect to the calculated $^3J_{X\text{-}ray}$ values Thus, dihedral angles H2-C2-C3-H3 and $H7_{eq}$-C7-C8-H8 (151 8° and -110 0° respectively) were rotated to dihedral angles (116° and -75° respectively) calculated from correspondent experimental $^3J_{HH}$ coupling constants This new geometry was than optimized by MM2 program with included chloroform dielectric constant ε=4 8 Obtained structure (Fig 1b) was used in further comparations and calculations as calculated solution state conformation of **1**

Solvent and temperature dependence

One characteristic of a molecule which exists in a single, a stable conformation is that the vicinal coupling constants remain invariant with respect to solvent and temperature changes[5] The proton NMR spectrum of azithromycin was recorded in various solvents and at different temperatures (Table 2) Increasing the temperature of a $CDCl_3$ solution from 293 to 323 K or changing the solvent not induced an averaging of the larger coupling constants They remain almost invariant except $^3J_{2\,3}$ Furthermore, the relative large chemical shift differences[13] between the diastereotopic protons at C7, C9, C14, C2" and C4' (Table 1) would indicate a

high conformational homogeneity From these results, it appears that azithromycin exists in solution as one major conformer, different from that found in the solid state, with the negligible participation of the other conformers However, the rise in $^3J_{2,3}$ indicates an increasing of "folded-out" (X-ray) conformation as temperature increases or the solvent is changed from $CDCl_3$ to more polar solvents

NOE

2D NOESY Experiments were performed on **1** in $CDCl_3$ solution The NOE results (Table 4) represent a spatial proximity for pairs of protons in the solution-state conformation The corresponding distances in Å between two protons obtained from the crystal structure and MM2 calculated solution structure of **1** are shown in parentheses

The crystal structure showed 176 contacts (88 proton pairs, time 2, an arbitrary cut-off of 3 Å) whereas the solution-state contained only 142 NOEs Of these 142 NOEs, 134 corresponded to interaction between protons less than 3 Å apart in the crystal structure, a remarkable level of agreement This is well illustrated by the intra-sugar, inter-sugar and sugar-lactone NOEs such as 1'-3', 1'-5', 3'NMe_2-4'a_X, 4"-3"Me, 1'-3"OMe, 1'-5, 1'-4Me, all of which corresponded to the interactions in the crystal structure of **1** The intra lactone NOEs as 11-10, 11-13 and 11-12Me confirmed that the C10-C13 portion of the lactone ring remains in similar conformation in solution as in the crystal state Also, the interactions observed in solution for the new ring-inserted 9aNMe group, NOE 9aNMe-10Me and 9aNMe-8, corresponded to the same position for this group in solution and in the crystal conformation However, 8 NOEs were observed for which no corresponding crystal structure contact existed, and 40 crystal structure contacts were found with no corresponding NOE contact Of the 8 outstanding NOEs 4 NOEs 2-3 and 7_{aX}-8Me and *vice versa*, were compatible with crystal structure (r(H,H)< 3 2 Å) and were excluded merely because of the arbitrary nature of the cut-off distance (3 Å) applied Of the 40 missing NOEs, 26 were missing due to technical difficulties (selective observation impossible) A further 6 were NOEs to methyl groups or methylene protons These later NOEs are inherently weak[14] and are frequently not observed

This left only 10 NOEs not observed but expected on the basis of the crystal structure - NOE 4-11, 7_{aX}-4Me, 2Me-3"OMe, 3'-3"OMe and 3'NMe_2-3"OMe and 4 NOEs observed but unexpected - NOEs between 2Me-4Me and between 4Me-3"OMe

These discrepancies were interpreted as follows

(i) 3'NMe_2-3"OMe and 3'-3"OMe In the crystal structure r(3'NMe_2,3"OMe) is 2 7 Å minimum (the minimum distance found by rotation of the methyl group) and r(3',3"OMe) is 2 3 Å, respectively In view of the fact that a NOE 1'-3"OMe was observed (r(1',3"OMe) is 3 0 Å) the lack of NOEs 3'NMe_2-3"OMe and 3'-3"OMe indicated that in solution these distances are >3Å Corresponding distances calculated for MM2 solution state conformation were 4 0 and 2 9 Å, minimum* respectively Furthermore, very long ^1H T_1 value for 3"OMe (0 56 s) was indicative of almost-free rotation in contrast to high rotational energy barrier (12 7 kcal/mol) calculated for 3"OMe in crystal structure

(ii) 4-11, 7_{aX}-4Me and 2Me-3"OMe More interestingly, the missing NOE 4-11 shows that the close, cross-ring approach of H11 and H4, for the crystal structure (r(11,4)=2 7 Å) so characteristic of "folded-out" conformation, was not present in solution Since the value of $^3J_{2\ 3}$=3 6 Hz indicated "folded-in" C3 to C5

* Here and later in the text "minimum" refers to the minimum distance found by rotation of the methyl group, thus, on average r(3',3"OMe)> 3 Å due to methyl group rotation

region in solution, we expected NOE between H11 and H3 In the 2D NOESY spectra no cross peak H-11/H-3 was observed Thus, more sensitive ^{1}H NOE difference experiments were used to determine spatially distance between these two protons Irradiation of H-3 and H-11 respectively, gave a medium-sized NOE, thus confirming presence of "folded-in" conformation in solution The weaker interaction from that we expected, indicated that the protons 11 and 3 are further in compound **1** than in **3** Molecular mechanics calculations for solution state conformation confirm the corresponding 1D results The calculated H11 to H3 distance was longer in **1** (3 1 Å) than in the same "folded-in" conformation of **3** (2 2 Å), probably due to the ring-enlargement of **1** In addition, the non-observation of NOEs 7_{ax}–4Me and 2Me-3"OMe supported the proposed "folded-in" conformation of **1** in solution Namely, the inward folding of the C3-C5 portion of the

Table 4 Qualitative NOE Data for Azithromycin **1**[a]

Contact Intraunits

Aglycone ring

2	3 (3 1/2 8), 4 (2 7/2 2), 2Me (2 5/2 4), 4Me (1 9/2 3)
4	3 (2 5/2 6), 5 (3 0/3 0), 7_{ax} (2.3/2.2), 4Me (2 4/2 4)
5	3 (2 5/2 4), 6Me (2 3/2 3)
7_{ax}	8Me (3 1/2 8)
7_{eq}	7_{ax} (1 8/1 8), 8 (2 9/2 7), 6Me[g](2 6/2 6), 8Me (2 4/2 3)
8	9_{eq}[b](2 7/2 6), 9aNMe (2 3/2 2), 6Me (2 1/2 2), 8Me[h](2 5/2 5)
9_{ax}	9_{eq}[b](1 8/1 8), 10 (2 0/2 1), 8Me[h](2.6/2 5), 10Me (2 7/3 0)
9_{eq}	9aNMe (2 6/2 4), 8Me (2 7/2 4), 10Me (2 4/2 4)
10	11 (2 7/2 5), 10Me[c](2 4/2 4), 12Me[c](2 2/2 2)
11	11OH (3 0/2 8), 13 (2 7/2 7), 12Me(3 0/2 9) 9aNMe (2 8/3.0)
11OH	12OH (2 7/2 4), 13 (2 7/2 6),
12OH	10Me[d](2 0/2 0), 12Me[d](2 2/2 3)
13	14_{eq} (2 6/2 5), 14Me (2 5/2 5)
14_{ax}	14_{eq} (1 8/1 8), 14Me (2 5/2 5), 12Me (2 3/2 2)
14_{eq}	14Me (2 5/2 5)
2Me	4Me (3 8/2 4)
10Me	9aNMe (2.2/2 4)

Desosamine

2'	$4'_{ax}$ (2 7/2 7)
3'	1' (2 5/2 6), $4'_{eq}$ (2 6/2 4), 5' (2 6/2 5),
$4'_{eq}$	$4'_{ax}$[e](1 8/1 8), 5' (2 5/2 5), 5'Me[e](2.7/2 4)
$4'_{ax}$	3'NMe_2[f](2 3/1 8)
5'	1' (2 5/2 4), 5'Me (2 5/2 4)

Cladinose

$2"_{ax}$	1" (2 4/2 4), $2"_{eq}$ (1 8/1 8), 4" (2 7/2 5), 3"Me (2 5/2 6)
$2"_{eq}$	1" (2 6/2 5), 3"Me[f](2 7/2 6), 3"OMe (2 1/2 2
4"	4"OH (3 0/2 8), 5"Me (2 6/2 6), 3"Me (2 4/2 5)
5"	5"Me (2 6/2 5)
3"Me	4"OH (2 5/2 7), 3"OMe (2 3/2 3)

Contact Interunits

Aglycone ring-Sugar rings

3	1" (2 2/2 5)
5	1' (2 2/2 3), 5" (2 1/2 1)
2Me	1" (2 1/2 2), $2"_{eq}$[f](2 3/2 8)
4Me	1' (2 7/2 3), 3"OMe (3 2/2 7)

Desosamine-Cladinose

1'	5" (2 2/2 8), 3"OMe (3 0/2 5)
5'	5" (2 4/2 8)
5'Me	5"Me[g](2 4/2 3)

[a] The NOEs observed only for azithromycin 1 are underlined while other NOEs are observed also for erythromycin A 2[5] Corresponding distances in Å between two protons (crystal structure/calculated solution state) for azithromycin 1 are in parentheses

[b,c d,e,f,g,h] Pairs of overlapping NOEs

Table 5 Experimental ^1H NMR relaxation times (T_1 in s, $CDCl_3$ solution) and calculated rotational energy barriers (calculated solution state conformation and X-ray conformation) for the methyl groups in **1**

Methyl	^1H T_1[a]/s	E^b(solution)	E^c(crystal)
3"OMe	0 56	A	D
14Me	0 46	B	A
5"Me	0 40	A	C
5'Me	0 39	A	A
3"Me	0 38	B	B
8Me	0 38	B	B
9aNMe	0 36	B	B
3'NMe_2	0 36	B	B
6Me	0 36	B	B
2Me	0 36	B	C
10Me	0 30	B	A
12Me	0 30	C	B
4Me	0 29	C	C

[a] Experimental results at 300 MHz for **1** in $CDCl_3$, T_1= spin-lattice relaxation time
[b] Theoretical calculations based on calculated solution state conformation of **1** The results are given in terms of energy ranges A(0-3), B(3-5), C(5-7) and D(>7 kcal mol^{-1})
[c] Theoretical calculations based on X-ray conformation of **1** Energy ranges are same as for solution state

lactone ring moves 4Me a more outside ring position, but forces it closer to the 2Me The 4Me is thus pushed into a more sterically congested environment This reorganization results in the NOEs 2Me-4Me and 4Me-3"OMe which interactions were not present in the crystal structure of **1**, but were found in the crystal structure of **3** Simultaneously, the rotation of 2Me group is less hindered due to an easing of the steric congestion between 2Me and 3"OMe that results in missing NOE 2Me-3"OMe, but was found in the crystal state of **1**

The motional properties of the methyl groups

^1H NMR Relaxation measurements (T_1 data) lead to the same conclusions as the NOEs experiments with the respect to the conformation of **1** in solution (Table 5) The "folded-out" solid conformation of **1** is characterized by a high calculated energy barrier to the rotation of 2Me, due to the close approach of H1"[5] The adoption of a "folded-in" conformation in solution reduced the mobility of 4Me, while lessening the restriction to the rotation 2Me The ^1H T_1 values for the 2- and 4-methyl groups were consistent with the above conclusions This value for 4Me (0 29 s) was shorter than was for 2Me (0 36 s) as would be expected for a pure "folded-in" conformation[10,15]

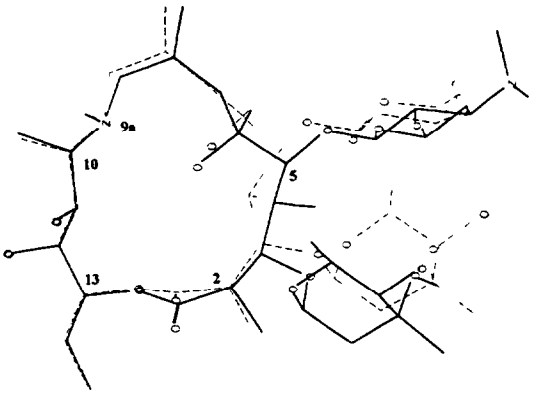
The remaining methyl groups had intermediate ^1H T_1 values in agreement with the intermediate energy barriers to rotation calculated for these methyl groups in the crystal structure and MM2 solution state conformation

Conclusions

A combination of NMR spectroscopy and molecular modelling techniques showed that the major conformation of **1** in solution differs from the crystalline state conformation

The solution state conformation of **1** is a C3 to C5 "folded-in" type, with H3 close to H11 (Fig 1b) similar to the crystalline state of dinthromycin **3** The contribution of the "folded-out" (X-ray) conformer in the solution

Fig 2 Superposition of solution state conformation (solid line) and crystal conformation (dotted line) of azithromycin 1



is low, but increases as the solvent is changed from $CDCl_3$ to the more polar solvents However, in the crystal 1 exist in a "folded-out" conformation with close cross-ring approach of H11 and H4 (Fig 1a) previously observed in the crystal state structure of erythromycin A hydroiodide dihydrate 2 The N9a to C13 region remains virtually unchanged Both the crystal structure and NMR studies show that the sugars in 1 have the same conformations and orientations as in erythromycin derivatives, but with longer distance between them in solution than in the crystal structure (Fig 2)

These results are the opposite of what is generally observed for erythromycin derivatives, which in solution retain predominantly the crystal state conformation

The theoretical calculations based upon molecular modelling techniques for comparation solution with crystalline state are in good agreement with NMR parameters such as ^3J, NOEs and ^1H T_1 data

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra were acquired at ambient temperature in 5 mm o d NMR tubes on Varian Gemini 300 spectrometer COSY spectra were acquired with sweep width of 3200 Hz into 1024 data points in F2 dimension The 90° pulse was 13 2 μs, the relaxation delay was 1 0 s and each FID was acquired with 8 scans and 2 dummy scans 256 Values of the evolution time were sampled but the data was zero filled to 1024 points in F1 prior to double Fourier transformation

The HETCOR spectra were acquired with sweep widths of 8403 4 Hz into 2048 points in F2, and 4500 5 Hz into 256 points in F1 dimension, respectively The 90° pulses for ^1H and ^{13}C were 13 2 and 14 6 μs, respectively Each FID was acquired with 256 scans and a relaxation delay of 1 0 s Experiments were acquired using standard Varian software

The phase-sensitive NOESY experiment was performed using the time-proportional-phase-increment method[16] FID were acquired (64 scans, 2 dummy scans) over 3300 3 Hz into a 2K data block for 512 increment values of the evolution time, t_1 The raw data were zero filled to a 2K*2K matrix and processed with a 0 1 Hz line-broadening function in both dimensions Experiments were performed with mixing time 0 45 s and the relaxation delay was 2 5 s

The ^1H T_1 experiment was conducted by using a standard inversion-recovery sequence (D_1-180°-VD-90°-FID) with the relaxation delay 4 s and averaging 32 scans into an 16K data block (acquisition time 3 2 s) The experiment was repeated for 9 values of the variable delay VD ranging from 0 0156 to 4 0 s The T_1 values were calculated by using standard Varian software The 180° pulse calibrated in $CDCl_3$ solution was 17 μs

The ^1H NOE difference spectra were acquired automatically using a modification of the method of Saunders[14] Typically, 8-10 irradiations were performed in one experiment using 4 dummy scans and 32 scans at each frequency The pulse sequence utilized a pre-irradiation delay (3 s), followed by a sub-saturating irradiation period (3-6 s), and then data acquisition with the decoupler gated off Difference spectra were obtained by the subtraction of the control (off-resonance irradiation) from every other spectrum

Molecular mechanics optimization of solution state conformation and calculations of rotational barriers for methyl groups were performed by using MM2 program[12] Rotational energy barriers for each methyl were obtained through relaxation of molecule (energy minimization) at methyl-molecule dihedral angle founded by rigid rotor approximation

Calculations of dihedral angles from ^3J values were performed using equation (1) by simple Turbo Pascal program based on graphic interpolation Empirical parameters P_1-P_6 used in equation (1) were there from original paper[8], χ was Huggins electronegativity of the substituents[17], and ξ was flag (+1 or -1) which represented orientation of the substituents

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