Conformational study of tylosin A in water and full assignments of ^{1}H and ^{13}C spectra of tylosin A in D_2O and tylosin B in $CDCl_3$

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Tylosin A and tylosin B

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Introduction

The first paper describing the use of a macrolide to prevent liver infections, and subsequent blood infections caused by the malaria parasite (*Plasmodium falciparum*) was published in 1995 where it was suggested, after a controlled phase II trial study, that azithromycin has the potential to be at least as effective as mefloquine and doxycycline. [1] However, randomized studies conducted at Mahidol University Hospital (Bangkok, Thailand) in 2005 concluded that azithromycin is not efficient as a single agent,[2] but that quinine, tafenoquine, artesunate and/or primaquine have additive, synergistic qualities when used with azithromycin. The study also reported that dihydroartemisinin tends to act as an antagonist.[2,3] Finally, the azithromycin-chloroquine combination was suggested as a potential alternative to the sulphadoxine-pyrimethamine treatment.[4]

McColm and McHardy reported in 1984 that tylosin A also has anti-malarial activity against the Liverpool strain of *P. falciparum* (IC_{50}=0.1 μg mL^{-1}). Novel C-9 oximes of tylosin B also show anti-malarial activity against chloroquine-resistant *P. falciparum* K1 malaria parasites.[5]

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The macrolide antibiotic tylosin A was first isolated from a strain of *Streptomyces fradiae* in 1961.[6] Its chemical structure was determined by several groups over the next decade.[7-11] Although 14-membered ring macrolides, such as erythromycin A were assigned to human use, tylosin A has been used in domestic animals, principally as an anti-inflammatory agent.[12] There are few adverse side-effects.

Tylosin A acts by binding to the 50S ribosomal subunit of susceptible bacteria. Resistance is mediated by the methylation of N1 of the nucleotide G748 within hairpin 35 of 23S rRNA, catalysed by the methyltransferase Rlm AII (formerly known as TlrB).[13] Resistance in the tylosin producing *Streptomyces fradiae* is mediated by TlrB, TlrD and TlrA. TlrB expression gives very minor resistance to tylosin by itself (MIC=2), and requires co-expression of TlrD (which N-methylates position A2058) for modest resistance to tylosin (MIC=256). High level tylosin resistance (>2000) is mediated by TlrA, which dimethylates position A2058. TlrA has the same enzymatic activity as ErmE.[14]

Remarkably, the first crystal structure of intact tylosin A has the drug bound to 50S ribosomal subunits of *Haloarcula marismortui*.[15] The X-ray structure of 5-*O*-mycarosyltylactone was determined earlier and represents the pioneering work in the crystallography of tylosin derivatives.[16]

There are reports of NMR assignments of tylosin A in aprotic solvents ($CDCl_3$, CD_3CN).[17,18] Simova *et al.*[18] reported that the spectral assignment for tylosin A in protic solvents (CD_3OD, D_2O) is very difficult because of the existence of three different tylosin A forms in CD_3OD (ketone, hemiacetal 1, hemiacetal 2) and two in D_2O (ketone, hydrate). They found that, in D_2O at 300 K, the ketone:hydrate ratio is 65:35. It is known that macrolides, such as erythromycins A and C often exist in several forms [19-22].

Tylosin B is a degradation product of tylosin A produced by acid hydrolysis.[23] (An alkaline degradation product of tylosin A, known as tylosinaldol, has also been found as a contaminant of medicinal tylosin intended for injection.)[24] Tylosin B (desmycosin) is important principally because it can be used as a starting material for the synthesis of more active derivatives. The following modifications of tylosin B have been reported: 1) replacement and modification of mycinose (labelled red); 2) modification of the dienone moiety (labelled pink); 3) modification of mycaminose (labelled blue); 4) modification of the C-3 hydroxyl group (labelled green) (Figure 1 B).[25]

(Figure 1 should be here)

The most successful derivative of tylosin B, tilmicosin, contains the substitution at position

20 (20-deoxo-20-(3,5-dimethylpiperidin-1-yl) desmycosin) [26] (Figure 2).

(Figure 2 should be here)

There have been attempts to determine the three dimensional structure of tylosin in aprotic solvents using molecular modelling and different force fields.[27,28] The molecular modelling data were combined with NMR data that included NOE experiments as well as the measurement of vicinal proton-proton coupling constants. These studies gave structures whose dominant conformation does not resemble that of the crystal structure of tylosin A.[29] We have previously shown that the larger macrolides do not behave as small molecules in standard molecular modelling calculations.[30-32]Overall, however, there is surprisingly little published work on the three-dimensional structure of tylosin A. In particular, the conformation of tylosin A in water has not been determined. In order to understand the conformation of tylosin A we carried out both molecular modelling and NMR study.

Results and Discussion

Determination of the carbonyl:hydrate ratio of tylosin A in deuterated water, using NMR spectroscopy

Simova *et al.*[18] reported that at 300 K tylosin A exists as a mixture of carbonyl and hydrate forms in a 65:35 ratio. We investigated the effect of temperature on this ratio in D_2O by NMR spectroscopy monitoring the integral of the aldehyde ^1H signal at 9.67 ppm, and comparing its value with that of proton H-11. Within the 298-338 K temperature range, an increase in temperature correlated with an increase of the aldehyde form. At 338 K, tylosin A exists mostly as an aldehyde (approximately 80 % of the total tylosin A). Here we see an interesting parallel with erythromycin A[19,33,34], which exists as a tautomeric mixture of keto and hemiacetal forms in aqueous solution (Figure 1).

The three-dimensional structure of tylosin A

An unconstrained conformational search on the aldehyde form of tylosin A in water was performed using molecular mechanics (Macromodel 8) with the MM2 force field.

As the starting point in the process of modelling the aldehyde form of tylosin A we have used the only available structure of tylosin A (in the form of co-crystal structure with 50 S ribosomal subunit of *Haloarcula marismortui*).[15] It is noteworthy that the crystal structure of tylosin A alone has not been published, although the drug was isolated for the first time in the mid-1960s.

Using Macromodel 8, we found the global minimum of tylosin A (Figure 3) 17 times (energy E=280.9 kJ mol^{-1}). The global minimum satisfies all of the NMR constraints derived from the ROESY experiments that were acquired to assist the molecular mechanics studies. The exceptions were H11-H13, $H_3$20-H4′, and $H_3$6″-H2″b (the ROESY correlation table is available in Supplementary Data as Table 1S). The $H_3$20-H4′ correlations are vanishingly small, while those of $H_3$6″-H2″s were attributed to spin diffusion.

The H3-H11 and H4-H11 contacts, (characteristic of 14- and 15-membered macrolides and necessary for grouping into various types of folded-in and folded-out structures), in the global minimum structure are 3.34 Å and 5.04 Å with no indications of folded-in or folded-out conformers. Other characteristic contacts for folded-in conformer (H3-H8, H8-H11) and H5-H6Me are 4.88 Å, 4.68 Å and 2.84 Å respectively, so tylosin A does not adopt a folded-in conformation. H5-H6Me Distance is short and characteristic of a folded-out conformation. The H4-H6Me, distance, characteristic of folded-in conformers is, in tylosin A, 4.83 Å, which indicates that tylosin A is certainly neither in a folded-in conformation nor in a folded-out one. It seems that the presence of double bonds inside the lactone ring, as well of certain sugar components make the conformation of tylosin A different from that of 14- and 15-membered macrolide antibiotics.

(Figure 3 should be here)

When the global minimum for tylosin A (green) was superimposed on the crystal structure obtained from co-crystallizing tylosyn A with *H. marismortui* ribosomes (pink), it was found that the two structures were almost identical, except for the positions of the mycinose rings that differ significantly (Figure 3). In particular, there is very good agreement in the macrolide ring. This is interesting because of the difficulty in obtaining agreements between the experimental and the modelled structures of azithromycin. This might be due to the fact that the double bonds in tylosin impose some rigidity, thus limiting the range of possible conformations.

2D ROESY NMR experiments on tylosin A

NOESY experiments are seldom appropriate for macrolides in solution. The NOE is normally positive for small molecules, goes through zero for medium-sized molecules (MW range 700-1500), and becomes negative for large molecules (MW>1500). The 2D-ROESY is therefore to be preferred in the present case (the molecular weight is 916 Da), although such spectra are often contaminated by TOCSY peaks. The mixing time was optimised

experimentally as 200 ms. The 2D ROESY NMR spectrum is presented in Figure 1S.

Superposition of structures of azithromycin and tylosin A

Baram *et al.*[35] published a crystal structure containing two molecules of azithromycin bound to the *Deinococcus radiodurans* ribosome. We have superimposed a tylosin molecule on top of these two azithromycin molecules, as shown in Figure 4. The structure of the two molecules of azithromycin were extracted from the reported co-crystal structure with the ribosome of *D. radiodurans,* while that of tylosin A was extracted from its co-crystal structure with the ribosome of *H. marismortui*. The operation was carried out using the MOE and the Macromodel 8 programmes. Figure 4 shows that a tylosin molecule has the size of two azithromycin molecules. It was suggested by Hansen *et al.* (2002) [36] that the mycinosyl moiety of tylosin interacts with A841 (A748, *E. coli* numbering) in domain II of the 50S ribosome. The mycarose unit is very important to the anti-bacterial activity; its removal reduces the anti-bacterial activity.[37] This is consistent with the observation that the mycarose sugar contributes up to 2/3 of the interaction surface energy, which is an important component of the binding free energy.[36]

(Figure 4 should be here)

Full ^{1}H and ^{13}C assignments of tylosin A in phosphate buffered solution (0.047 mol L^{-1}, uncorrected pD=7)

The proton and carbon assignments of tylosin A were based on ^{1}H, ^{13}C{^{1}H} DEPT, COSY, ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HSQC.

The assignments are shown in Table 1. Detailed explanation of the assignment procedure together with the spectra used in the analysis is available in Supplementary Material.

(Table 1 should be here)

^{1}H and ^{13}C assignments of tylosin B (desmycarosyl tylosin) in $CDCl_3$ are shown in Table 2. Detailed description of the assignment procedure, as well as the spectra used is placed in Supplementary material.

(Table 2 should be here)

Experimental

Synthesis of tylosin B (desmycarosyl tylosin)

The mixture of tylosin A tartrate (1.0000 g) and 0.2 M HCl (20 mL) was stirred for 4 h. After

the reaction was finished, extraction with chloroform (4x15 mL) was performed in order to remove mycarose. The pH of the solution was adjusted to 8.0 using a saturated solution of sodium bicarbonate and a universal indicator, and was extracted with chloroform (4x15 mL). The extract was dried over anhydrous magnesium sulphate, the solid removed by filtration, and the filtrate evaporated to dryness. White crystalline tylosin B was obtained (0.5101 g, yield 70.45 %). Mp. 113-116 °C. The compound was identified using a combination of ^{1}H, $^{13}C\{^{1}H\}$ ^{1}H-^{1}H DQF-COSY, ^{1}H-^{1}H ROESY, ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HMQC and low and high resolution mass spectrometry. m/z (ES+): 772.6; HRMS (EI): m/z calculated for $C_{39}H_{66}O_{14}N_1$: 772.4478. Found 772.4467.

Molecular modelling

Tylosin A was constructed from the crystal structure of tylosin A[15] using Macromodel 8 software.[38] The conformational analysis was performed *via* the Monte Carlo/multiple minimum (MC/MM) approach, to identify the lowest energy conformation in solution.[39] The solute was described using the all-atom MM2 potential.[40] The effect of the solvent was incorporated into the MC/MM calculations using the generalised Born/surface area (GB/SA) continuum solvent model for water.[41] Cut-offs of 12.0 Å and 7.0 Å were employed for electrostatic and van der Waals non-bonded interactions, respectively. The MC simulation involved 10^4 steps at 300 K, applied to all rotatable bonds, with random torsional rotations of up to ±180°. This was combined with 10^3 steps of energy minimization. All conformational calculations were performed using the Macromodel 8.0 and BatchMin suite of programs.[38]

NMR analysis

Tylosin A tartarate was obtained from Aldrich and used without further purification while tylosin B was synthesized as previously described. All measurements were carried out in deuterated water or chloroform. A 0.047 mol L^{-1} phosphate buffer was used in the former case that give a pD of 7 (values provided without correction). The measurements were performed using BrukerAvance 400 MHz and 500 MHz spectrometers (Karlsruhe, Germany). **NMR experiments of tylosin A in phosphate buffered D_2O (pD=7, values provided without correction).** The operating frequency for the ^{1}H measurements was 400.132 MHz; the spectral width (SWH) was 8223.685 Hz, the acquisition time (AQ) 3.98 s, the high-power pulse (P1) 90˚ pulse was 7.79 μs, 65536 complex data points were collected (TD). The number of scans was 16, the relaxation delay (d1) was 2.4 s. The temperature was 303.7 K.

Exponential functions were used with a line broadening (LB) of 0.3 Hz. For the $^{13}C\{^{1}H\}$ spectrum, the operating frequency was 125.77 MHz, the P1=8.80 μs, AQ=1.1 s, 2 dummy scans were used, SWH=29761.904 Hz, TD=65536; for the DEPT 90 spectrum, the operating frequency was 125.77 MHz, P1=8.80 μs, AQ=1.1 s, dummy scans=4, SWH=29761.904 Hz, TD=65536; for DEPT 135 spectrum, operating frequency was 125.77 MHz, P1=8.80 μs, AQ=1.1 s, dummy scans=4, SWH=29761.904 Hz, TD=65536. Exponential function was used as a window function for processing ^{13}C spectrum with LB=1.50 Hz. The same window function was used for DEPT 90 and DEPT 135 processing, but LB values in both cases were 1.00 Hz. In case of the COSY spectrum, the operating frequency was 400.132 MHz, AQ=0.25 s, SWH=4157.4 Hz, TD=2048x512, and QSINE window functions using a Fourier number (SI) of 4096 and 2048 for the direct and indirect dimensions were performed, respectively, with SSB=1. The acquisition parameters for the HSQC spectrum were AQ=0.15 s, SW(F1)=165.6 ppm, SWH(F2)=3378.4 Hz, TD=1024x256. The Echo-Antiecho procedure was used to produce phase sensitive data (FnMODE) with operating frequencies for ^{1}H and ^{13}C equal to 400.132 MHz and 100.62 MHz, respectively. The processing parameters for HSQC were: window function-QSINE, SI=4098x1024, SSB=2. Regarding the HMBC, the acquisition parameters were: AQ=0.61 s, SW(F1)=221.8 ppm, SWH (F2)=3378.4 Hz, TD=4096x128 with operating frequencies for ^{1}H and ^{13}C equal to 400.13 MHz and 100.61 MHz, respectively. The ROESY experiments were recorded using the following acquisition parameters: operating frequency 400.13 MHz, TD=2048x512, AQ=0.23 s, D1=2 s, P15=200 ms, FnMODE-States-TPPI; and the following processing parameters: SI=2048x1024, window function was QSINE, SSB=2.

NMR experiments of tylosin B in deuterated chloroform. The operating frequency for ^{1}H measurements was 300.13 MHz; spectral width was SWH=6172.839 Hz, acquisition time AQ=5.3 s, high-power pulse (P1)=8.3 μs, data points (TD)=65536, number of scans=16, relaxation delay D1=2.4 s; T=294.4 K. Exponential function was used as a window function for processing with LB=0.3 Hz. For the ^{13}C spectrum, the operating frequency was 75.48 MHz, P1=7.80 μs, AQ=1.74 s, dummy scans=4, SWH=18832.393 Hz, TD=65536; for DEPT 90 spectrum, the operating frequency was 75.48 MHz, P1=7.80 μs, AQ=1.82 s, dummy scans=4, SWH=17985.611 Hz, TD=65536; for DEPT 135 spectrum, operating frequency was 75.48 MHz, P1=7.80 μs, AQ=1.82 s, dummy scans=4, SWH=17985.611 Hz, TD=65536. Exponential function was used as a window function for processing ^{13}C spectrum with LB=2.00 Hz. The same window function was used for DEPT 90 and DEPT 135 processing

and the same LB value (LB=2.00 Hz). In case of COSY spectrum, operating frequency was 300.13 MHz, AQ=0.30 s, SWH=3360.215 Hz, TD=2048x128, and QSINE window function was used for processing with SI=4096x1024 and SSB=1. The acquisition parameters for the HMQC spectrum were: AQ=0.15 s, SW(F1)=165.6 ppm, SWH(F2)=3360.215 Hz, TD=1024x128, FnMODE-QF with operating frequencies for ^{1}H and ^{13}C equal to 300.13 MHz and 75.47 MHz, respectively. Processing parameters for HMQC were: window function-QSINE, SI=4096x1024, SSB=1 and MC2-QF. Regarding the HMBC, the acquisition parameters were: AQ=0.64 s, SW(F1)=222.3 ppm, SWH (F2)=3205.128 Hz, TD=4096x128 with operating frequencies for ^{1}H and ^{13}C equal to 300.13 MHz and 75.48 MHz, respectively.

Conclusions

We report the full ^{1}H and ^{13}C NMR assignments on tylosin A in deuterated water, as well as those of tylosin B in deuterated chloroform. NMR investigations on the equilibrium of carbonyl to hydrate ratio shows that the percentage of the aldehyde form increases with the rise of temperatures. Molecular mechanics calculations performed using the Macromodel 8 package gave, as a global minimum, a conformation of tylosin A which satisfies almost all 2D ROESY NMR constraints, except those of H11-H13, $H_3$20-H4', and $H_3$6"-H2"b. Keeping in mind that $H_3$20-H4' is vanishingly small and that $H_3$6"-H2"s is probably due to spin diffusion, we can say that modelling represents an excellent tool for the prediction of the conformation of tylosin A in aqueous solution. It was found that the predicted structure is almost the same as the crystal structure (differences occurred only in the positions of the mycinose rings). Two azithromycin molecules are almost completely superimposable on one tylosin A structure, which suggests that tylosin A can potentially be used as anti-malarial.

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Table 1. The full ^{1}H and ^{13}C NMR assignments of tylosin A in phosphate buffered D_2O (pD=7, values provided without correction)

Position	Multiplicity	^{1}H (ppm)	J_{HH} (Hz)	^{13}C (ppm)	HMBC connectivities ($^{13}C \rightarrow {}^{1}H$)
1	-	-	-	181.3	H2a, H2b
2	m m	2.18 2.67	- -	42.5	$H_3$18
3	m	3.83	-	71.3	$H_3$18
4	m	1.67	-	43.6	-
5	m	3.65	-	83.6	$H_3$18
6	m	1.94	-	34.0	$H_3$21
7	m m	1.50 1.90	- -	34.6	$H_3$21
8	m	2.55	-	48.0	$H_3$21
9	-	-	-	212.2	H10, H11, $H_3$21
10	d	6.59	15.5	121.6	-
11	d	7.32	15.5	152.5	$H_3$22
12	-	-	-	139.1	H11, $H_3$22
13	dd	5.96	10.4	146.8	H10, H11, $H_3$22
14	m	3.14	-	47.6	-
15	dt	4.89	10.4; 2.6	78.8	$H_3$17
16	m m	1.85 1.60	- -	27.6	$H_3$17
17	t	0.94	7.3	11.8	-
18	d	0.98	3.9	11.3	-
19	m m	2.93 2.60	- -	46.6	$H_3$21
20	s	9.67	-	210.9	-
21	d	1.24	6.5	19.9	H7
22	s	1.87	-	18.2	H11
23	dd m	4.00 3.71	10.2; 3.4 -	72.3	H14
1′	d	4.45	7.2	104.8	H2′
2′	m	3.73	-	77.2	$H_3$6′
3′	m	3.79	-	46.6	$H_6$7′/8′
7′/8′	s	3.06	-	43.9	$H_6$7′/8′
4′	m	3.69	-	75.5	$H_3$6′
5′	m	3.42	-	76.7	$H_3$6′
6′	d	1.36	8.6	20.7	H2′
1″	d	5.19	2.5	99.3	$H_3$6″, $H_3$7″
2″	m dd	2.05 1.99	- 4.1	42.9	$H_3$7″
3″	-	-	-	72.7	H1″, H2″a, H2″b, $H_3$6″, H_3 7″
4″	d	3.23	9.1	77.9	$H_3$6″, $H_3$7″
5″	m	3.86	-	69.8	H4″, $H_3$6″
6″	d	1.33	2.7	20.1	H4″
7″	s	1.28	-	28.0	H4″
1‴	d	4.62	8.1	103.5	H2‴, H3‴

2‴	dd	3.16	8.4;2.8	83.1	H3‴, $HOCH_3$2‴
$OCH_3$2‴	s	3.47	-	60.9	H2‴, H4‴, H5‴
3‴	t	3.95	2.7	81.9	$HOCH_3$3‴, H2‴, H4‴’
$OCH_3$3‴	s	3.57	-	64.5	H3‴, H4‴, H5‴
4‴	dd	3.34	9.8;2.7	72.7	H3‴, H6‴
5‴	m	3.75	-	72.2	H1‴, H4‴, H6‴
6‴	d	1.24	6.5	19.6	-

Table 2. Multiplicities, proton and carbon chemical shifts and HMBC connectivities ($^{13}C \rightarrow ^{1}H$) for tylosin B (desmycosine tylosin) in $CDCl_3$

Position	Multiplicity	^{1}H (ppm)	^{13}C (ppm)	HMBC connectivities ($^{13}C \rightarrow ^{1}H$)
1	-	-	173.9	H2a, H2b, H3
2	m d	2.45 1.94	39.4	$H_3$18
3	d	3.84	70.1	H2a
4	m	1.56	75.1	-
5	m	3.71	81.9	$H_3$18, H1′,H2′
6	m	2.27	30-35*	$H_3$21
7	m m	1.47 1.64	30-35*	$H_3$21
8	m	2.57	44.7	$H_3$21
9	-	-	203.2	H10, H11
10	d	6.25	118.4*	-
11	d	7.30	148.1	H13, $H_3$22
12	-	-	134.9	H10, H11, $H_3$22
13	d	5.91	142.3	H11, $H_3$22, H23a, H23b
14	m	2.92	45.0	H23a, H23b
15	t	4.98	75.1	H16b, $H_3$17, $H_3$22, H23a
16	m m	1.86 1.53	25.5	$H_3$17
17	t	0.94	9.7	H16a
18	d	1.1	9.0	-
19	m m	2.93 2.36	43.7	$H_3$20
20	s	9.70	203.0	H19a, $H_3$21
21	d	1.21	17.4	H7a, H7b
22	s	1.79	13.0	H11, H13, H16b
23	dd m	4.01 3.57	69.0	H2‴
1′	d	4.25	104.0*	H5, H3′, H4′
2′	m	3.60	71.0	H3′, H4′, H6 7′/8′
3′	m	2.47	70.6	H4’, H5’, H6 7’/8’
4′	m	3.52	75.1	H2′, H36′
5′	m	3.30	73.3	-
6′	d	1.26	17.8	H2′
7′/8′	s	2.50	41.7	H3′, $H_3$6′, H_6 7′/8′
1‴	d	4.56	101.1	H15, H23a, H23b, H2‴, H3‴
2‴	m	3.02	81.9	H3‴, H4‴
OCH_3-2‴	s	3.49	59.7	H2‴, H3‴, H4‴
3‴	m	3.75	79.8	H1‴
OCH_3-3‴	s	3.62	61.8	H_3(OCH_3-2‴), H3‴
4‴	m	3.27	72.6	H2‴, H5‴
5‴	-	3.75	70.8	H1‴, H3‴, $H_3$6‴
6‴	d	1.26	17.8	H2‴

* Signals are very broad due to chemical exchange. In the case of C1′ and C10, it was possible to see the apex of the peak (values are presented in the table). Regarding C6 and C7, however, peaks are merged and broad, so it is not possible to determine the position of the apex.

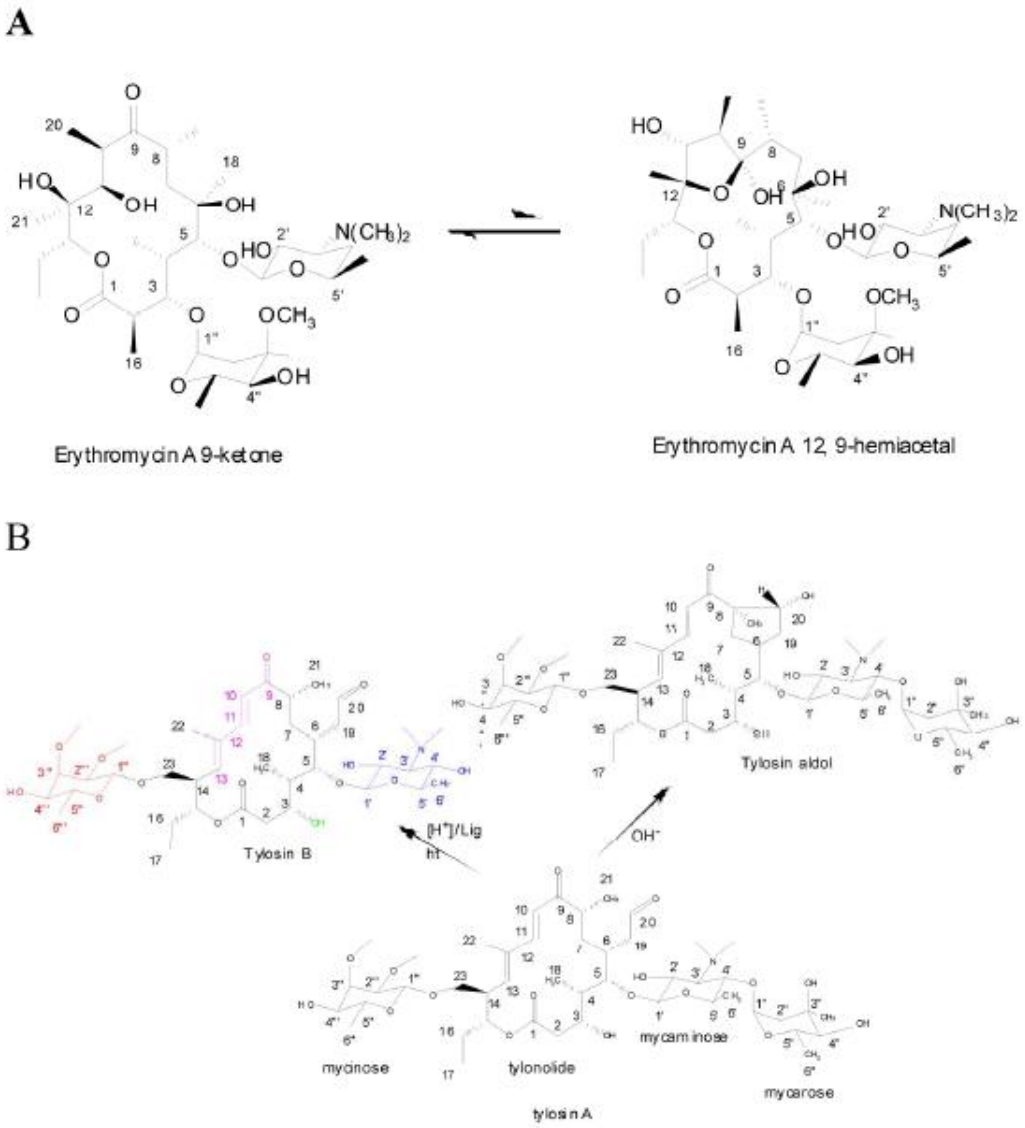

Figure 1. Different forms of erythromycin A and B (A), as well as of tylosin A in aqueous solutions (B).

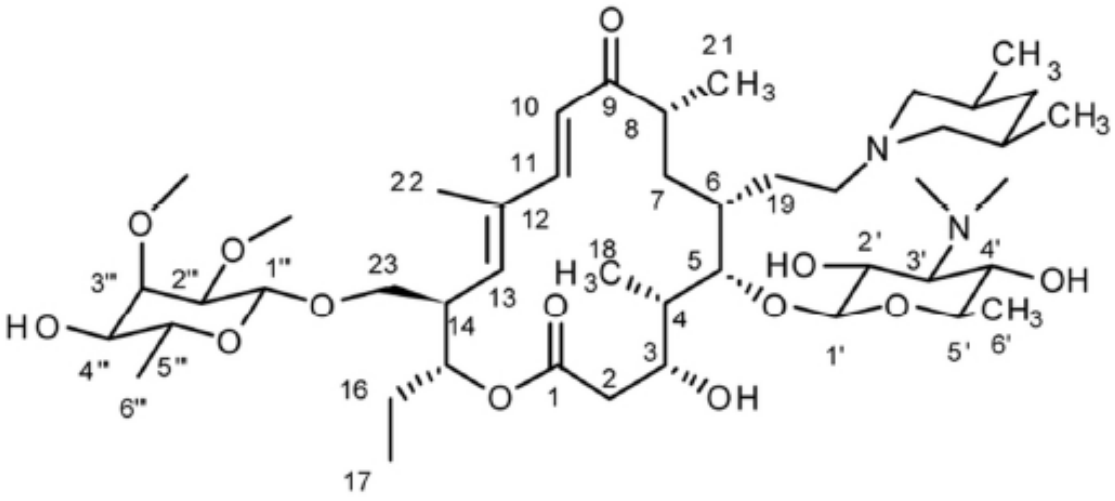

Figure 2. The structure of tilmicosin.

A

B

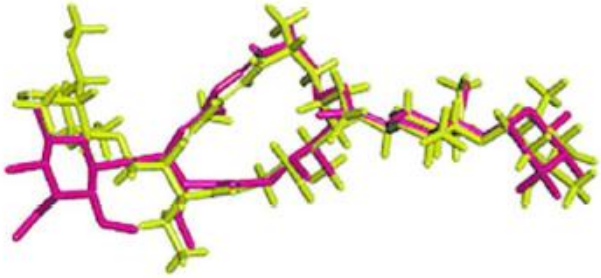

Figure 3. The global minimum of tylosin A obtained using Macromodel 8 can be seen in (A), while (B) shows the superposition of several tylosin A structures obtained by modelling (green) with the one extracted from the co-crystal structure of tylosin A with the 50S ribosomal subunit from *H. marismortui* (pink).

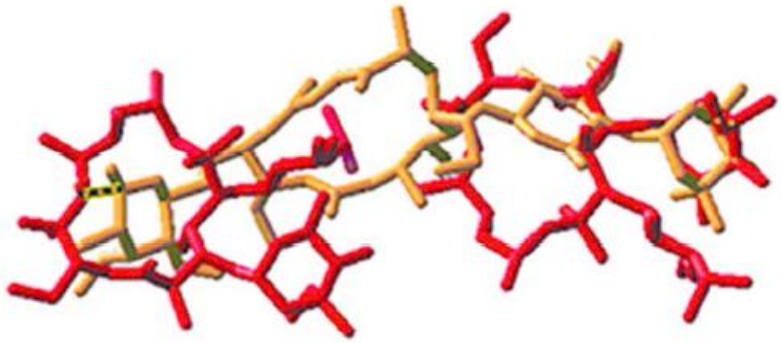

Figure 4. Superimposed structures of two azithromycin molecules with one of tylosin A. the yellow molecule is tylosin A and the red one is azithromycin.

Conformational study of tylosin A in water and full assignments of ^{1}H and ^{13}C spectra of tylosin A in D_2O and tylosin B in $CDCl_3$

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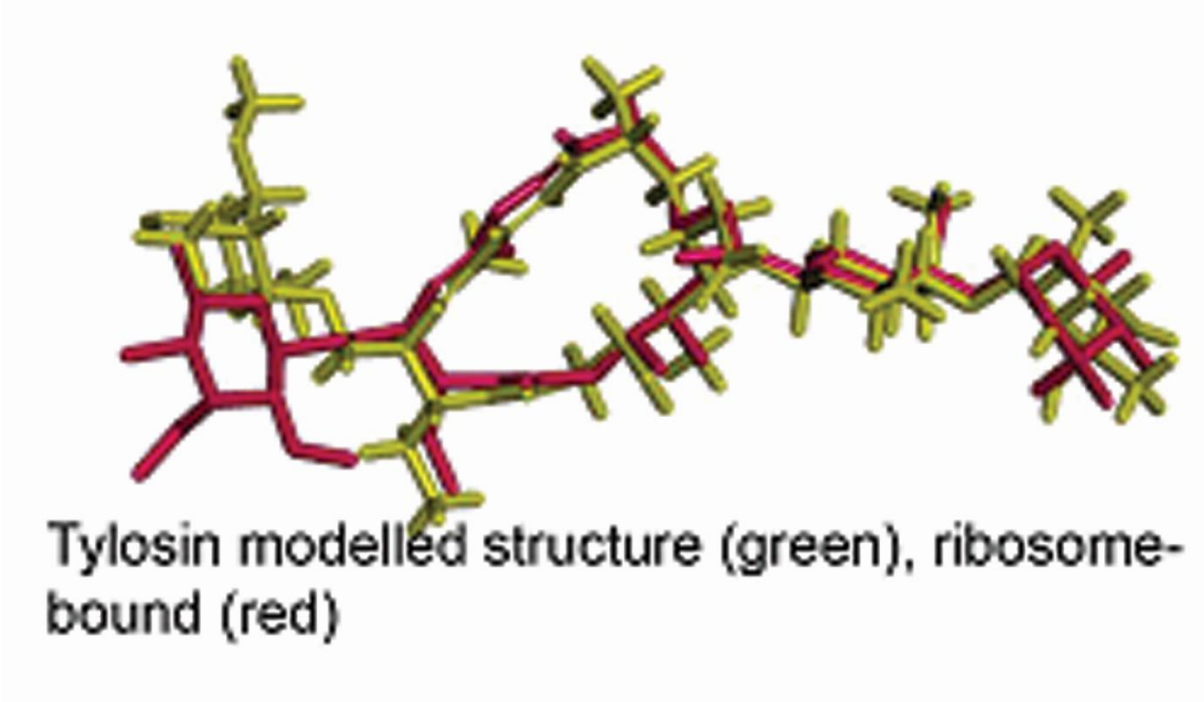

Full ^{1}H and ^{13}C NMR assignments of the macrolide antibiotic tylosin A in deuterated water are reported for the first time. A combination of molecular modelling and ROESY NMR experiments has been used to investigate the three-dimensional structure of this macrolide in solution. Its antibacterial activity and of that the closely related tylosin B have been determined *in vitro*