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Conformational Analysis of Dirithromycin and Epi-Dirithromycin[1]

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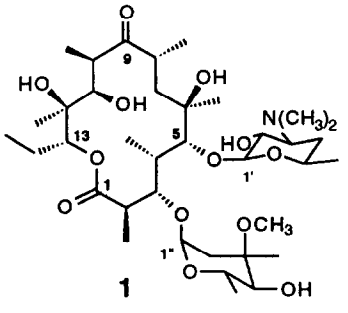
Abstract: The solution conformations of dirithromycin (3) and epi-dirithromycin (4) were determined by NMR spectroscopy and molecular mechanics calculations. Dirithromycin exists in a "folded-in" conformation which is similar to its crystalline conformation, while epi-dirithromycin exists in a "folded out" conformation which is similar to the crystalline conformation of erythromycin A hydroiodide dihydrate. Included is a discussion of the kinetically versus thermodynamically controlled formation of the remote aminal stereocenter.

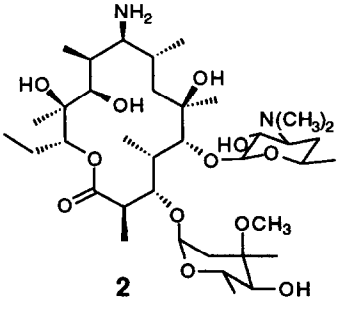
INTRODUCTION

Since its discovery in 1952, Erythromycin A (**1**) has been the most widely effective of the macrolide antimicrobial agents.[2] (9S)-Erythromycylamine (**2**), formally the reductive amination product of **1**, is a well known derivative of erythromycin which shows excellent antimicrobial activity but is poorly absorbed upon oral administration.[3,4] (9S)-Erythromycylamine reacts with aromatic aldehydes and ketones to form Schiff bases which can be reduced with sodium borohydride to give secondary amine derivatives. Neither the imines nor the secondary amines provided any improvement in the oral availability of **2**.[3] In contrast, aliphatic aldehydes react with **2** to give 9-N,11-O-oxazine derivatives.[4] Dirithromycin[5] (**3**), which affords high levels of the antibiotic in tissue after oral administration, is the 9-N,11-O-oxazine derived from **2** (Scheme 1) and 2-methoxyethoxy-acetaldehyde (**5**). The existence of the C-9, C-11 aminal bridge with an (R) configuration at the newly formed stereocenter at C-22 was confirmed by X-ray analysis.[6] The isomerization of pure **3** has been reported to occur upon dissolution in several different solvents.[7] The structure of the minor isomer was concluded to be the



1

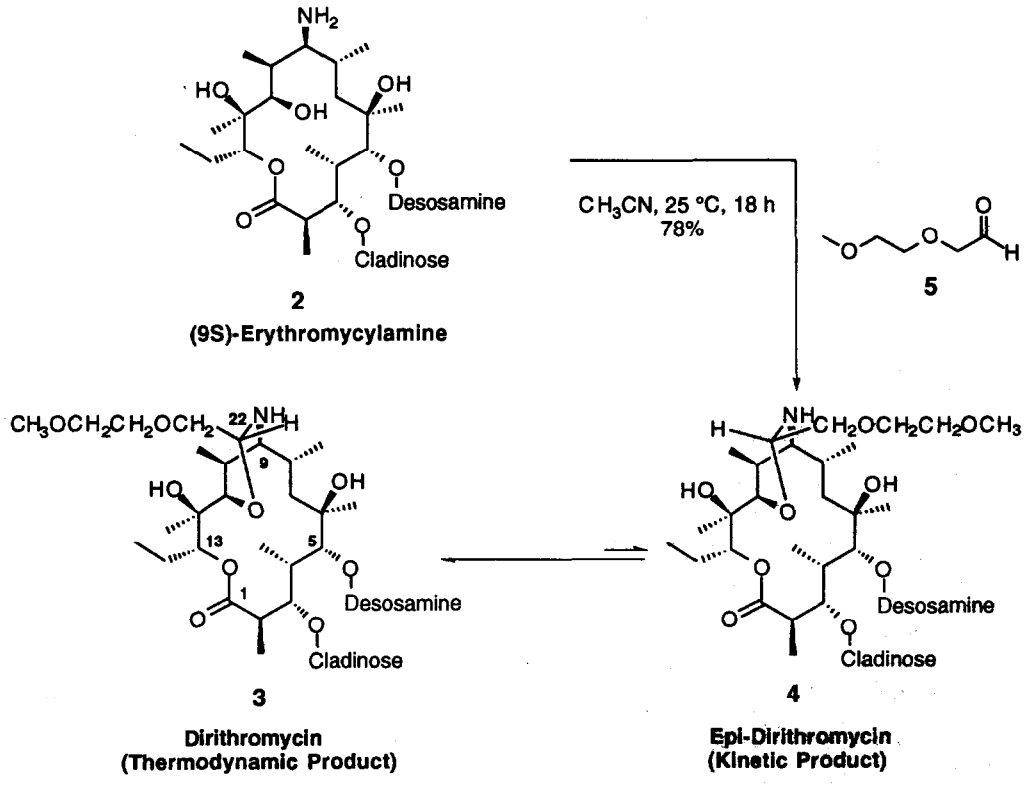
Erythromycin A



2

(9S)-Erythromycylamine

SCHEME 1



oxazine with (S)-configuration of the aminal center (**4**). However, this determination was based upon the comparison of NMR data obtained on a mixture of isomers. The unambiguous assignment of the isomerization product was hampered by the inability to isolate pure epimerized product.

Dirithromycin is prepared by condensing **5** with (9S)-erythromycylamine in acetonitrile.[5,8] Rigorous HPLC analysis[9] of this condensation reaction mixture shows the kinetic formation of a product which slowly equilibrates to the thermodynamically more stable **3** which crystallizes from solution. The structure of this kinetic product proved to be that of the dirithromycin C-22 epimerization product, epi-dirithromycin (**4**). Pure **4** was prepared by condensing **2** with an excess of **5** in diethyl ether. The kinetic product crystallized from ether as it was formed. Presumably, oxazine formation is the result of intramolecular addition of the C-11 hydroxyl group on an intermediate imine. The fact that aminal formation kinetically gives a single diastereomer, indicates that there is a formidable facial bias in the cyclization of the intermediate imine.

This work had three main objectives: (i) the complete and unambiguous assignment of the kinetic product as the C-22 epimer of dirithromycin; (ii) the determination of the solution conformation of both dirithromycin and epi-dirithromycin for comparison with each other as well as to the X-ray structure of dirithromycin; (iii) the development of a mechanistic rationale for the kinetic formation of epi-dirithromycin using the conformational information.

RESULTS and DISCUSSION

Analysis of ^1H and ^{13}C NMR Spectra

Recently, Counter and his colleagues reported the ^1H and ^{13}C NMR chemical shift assignments of **3**, however coupling constant information was not made available.[5] Here, the ^{13}C and ^1H NMR chemical shift assignments as well as coupling constant values for **3** in $CDCl_3$ are listed in Tables 1 and 2.[10] First-order coupling constant data for **3** was obtained using a ^1H 2D J-resolved experiment.[11] The J-resolved experiment allowed accurate determination of coupling constants even for signals that overlapped. However, in some cases of severe overlap, the coupling constants were obtained from 1D spectra where selective decoupling was used to simplify the specific region of interest.

Previously, Firl and Prox reported the partial assignment of the ^1H and ^{13}C NMR chemical shifts of **3** and **4**.[7] These assignments were complicated by the fact that **4** could only be investigated as a minor component in a mixture with **3**. The unambiguous structural assignment of **4** was made possible by the isolation of the pure epimer. The ^1H and ^{13}C NMR spectral assignments of **4** were assigned using a variety of two-dimensional NMR techniques. The ^{13}C and ^1H NMR chemical shift assignments as well as coupling constant values for **4** in $CDCl_3$ are listed in Table 1 and 2.[10]

TABLE 1: ^{13}C Chemical Shifts for Dirithromycin (**3**) and Epi-Dirithromycin (**4**) in $CDCl_3$.

DIRITHROMYCIN				EPI-DIRITHROMYCIN			
Carbon	δ (ppm)	Carbon	δ (ppm)	Carbon	δ (ppm)	Carbon	δ (ppm)
1	176.9	22	82.6	1	175.1	22	82.4
2	44.3	23	72.7	2	44.7	23	71.5
3	76.6	24	70.9	3	80.1	24	73.0
4	44.4	25	71.8	4	39.1	25	70.4
5	78.9	26	58.9	5	83.9	26	49.4
6	74.4	1'	100.8	6	73.9	1'	103.1
7	39.1	2'	70.9	7	34.3	2'	70.6
8	29.2	3'	64.8	8	33.0	3'	65.2
9	65.6	4'	28.7	9	63.7	4'	28.2
10	27.4	5'	69.3	10	29.3	5'	68.7
11	72.6	6'	21.0	11	69.9	6'	21.4
12	74.2	NMe_2	40.3	12	73.5	NMe_2	40.2
13	76.2	1"	94.2	13	75.7	1"	96.3
14	21.3	2"	34.3	14	20.9	2"	34.8
15	11.1	3"	72.7	15	10.7	3"	72.4
16	12.8	4"	78.3	16	16.3	4"	77.7
17	8.9	5"	65.8	17	9.3	5"	65.2
18	24.6	6"	18.3	18	27.3	6"	18.6
19	20.7	7"	21.8	19	21.3	7"	21.5
20	14.0	8"	49.1	20	19.3	8"	59.1
21	14.7			21	15.3		

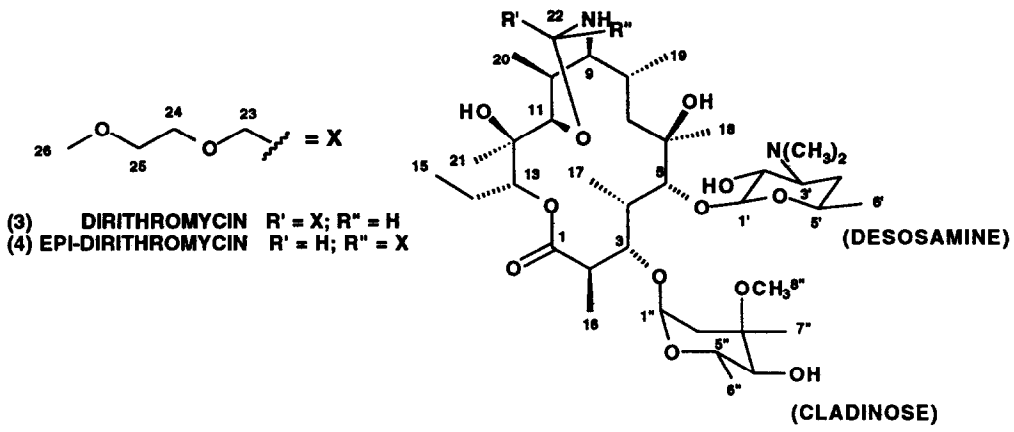
TABLE 2: ^{1}H Chemical Shifts and ^{1}H-^{1}H Coupling Constants for Dirithromycin (3) and Epi-Dirithromycin (4) in $CDCl_3$.

	DIRITHROMYCIN		EPI-DIRITHROMYCIN	
POSITION	δ (ppm)	$^{3}J_{H,H}$ (Hz)	δ (ppm)	$^{3}J_{H,H}$ (Hz)
2	2.68	J = 1.6 & 7.3 Hz	2.85	J = 9.0 & 7.1 Hz
3	4.00	J = 1.6 & 2.3 Hz	4.22	broad doublet J = 9.0 Hz
4	1.79	J = 2.3, 3.5 & 6.8 Hz	1.91	J = 7.0, & 7.9 Hz
5	3.95	J = 3.5 Hz	3.60	J = 7.9 Hz
7	1.35/1.65	multiplet[A]	1.40/1.57	multiplet[A]
8	2.10	multiplet[A]	2.05	multiplet[A]
9	2.15	$J_{8,9}$ = 9.6 Hz	2.55	$J_{8,9}$ = 1.1 Hz
10	1.78	J = 7.0 & 1.1 Hz	1.95	J = 0.9 & 7.0 Hz
11	3.23	J = 1.1 Hz	3.28	J = 0.9 Hz
13	4.91	J = 10.0 & 2.5 Hz	5.07	J = 10.9 & 2.1 Hz
14a	1.39	J = 14.3, 7.6 & 2.5 Hz	1.41	J = 14.4, 2.1 & 7.3 Hz
14b	1.89	J = 14.3, 7.6 & 10.0 Hz	1.89	J = 14.4, 10.9 & 7.3 Hz
15	0.87	J = 7.6 Hz	0.80	J = 7.3 Hz
16	1.18	J = 7.3 Hz	1.17	J = 7.1 Hz
17	1.09	J = 6.8 Hz	1.09	J = 7.0 Hz
18	1.12	singlet	1.32	singlet
19	1.33	J = 5.5 Hz	0.98	J = 7.0 Hz
20	1.14	J = 7.0 Hz	1.07	J = 7.0 Hz
21	1.08	singlet	1.08	singlet
22	4.57	broad singlet	4.72	J = 6.9 & 3.6 Hz
23	3.58	multiplet[A]	3.56/3.43	multiplet[A]
24	3.52	multiplet[A,B]	3.35/3.64	multiplet[A,B]
25	3.60/3.75	multiplet[A,B]	3.60	multiplet[A,B]
26	3.60	singlet	3.30	singlet
1'	4.78	J = 7.2 Hz	4.41	J = 7.2 Hz
2'	3.28	J = 7.2 & 10.3 Hz	3.25	J = 7.2 & 10.2 Hz
3'	2.51	J = 10.3, 12.1 & 3.7 Hz	2.45	J = 12.2, 10.4 & 3.7 Hz
4'eq	1.64	J = 14.5, 3.7 & 1.8 Hz	1.69	J = 14.5, 3.7 & 1.8 Hz
4'ax	1.26	J = 14.5, 12.1 & 11.5 Hz	1.20	multiplet[A]
5'	3.61	J = 11.5, 1.8 & 6.3 Hz	3.50	multiplet[A]
6'	1.24	J = 6.3 Hz	1.21	J = 6.4 Hz
NMe_2	2.27	singlet	2.30	singlet
1"	5.20	J = 5.0 Hz	4.90	J = 4.9 Hz
2"ax	1.56	J = 15.3 & 5.0 Hz	1.60	J = 15.0 & 4.9 Hz
2"eq	2.40	J = 15.3 Hz	2.40	J = 15.0 Hz
4"	3.01	J = 9.4 & 9.0[C] Hz	3.05	J = 9.8 & 9.0[C] Hz
5"	4.00	J = 9.4 & 6.3 Hz	4.05	J = 9.8 & 6.3 Hz
6"	1.24	J = 6.3 Hz	1.32	J = 6.3 Hz
7"	1.27	singlet	1.23	singlet
8"	3.35	singlet	3.30	singlet

[A] Overlapping resonance located by 2D ^{1}H Methods. Coupling constants could not be accurately measured.

[B] Resonance assignments may be interchanged.

[C] The additional coupling constant value (9.0 Hz) represents a coupling between the 4" proton and 4"OH.



Conformational Analysis

The solution structures of various erythromycin derivatives and their aglycones have been studied using a variety of techniques including NMR spectroscopy.[12] In 1987 Everett used ^{1}H and ^{13}C NMR experiments to determine the solution conformation of **1**.[13] In contrast to previous studies, Everett proved that the macrocyclic lactone of **1** exists in rapid conformational equilibrium between two different conformations. The major conformation found in solution, designated the "folded-out" conformation,[13] is very similar to the crystalline conformation of erythromycin A hydroiodide dihydrate.[14] The minor conformation, termed the "folded-in" conformation,[13] results from an inward folding of the C-3 to C-5 portion of the macrocyclic lactone ring and is similar to the crystalline conformation of dirithromycin.[6,15] The "folded-out " conformation is characterized by a close cross-ring proximity of C-4 and C-11, and relatively large values for $J_{2,3}$ (≈10 Hz) and $J_{4,5}$ (≈8 Hz).[16] The "folded-in" conformation is characterized by a close cross-ring proximity of C-3 and C-11, and relatively small coupling constants for $J_{2,3}$ (≈2 Hz) and $J_{4,5}$ (≈3 Hz). Following this pioneering report, the conformational analyses of several derivatives of erythromycin A including **2** have been reported.[17] In contrast to **1**, (9S)-erythromycylamine, which exists in rapid equilibrium between the two different macrocyclic conformations, prefers the "folded-in" conformation.[18]

Initial analysis of the ^{1}H NMR $^{3}J_{H,H}$ values indicated that **3** and **4** were conformationally quite different. A comparison list of the $^{3}J_{H,H}$ values and the corresponding calculated dihedral angles for vicinal proton pairs is outlined in Table 3. The dihedral angles were calculated from the Karplus equation by the method of Haasnoot.[19] While many of the coupling constants are quite similar for both molecules (especially the C-9 to C-11 portion and the sugar rings), the $J_{2,3}$, $J_{4,5}$ and $J_{8,9}$ are very different for each compound. In addition, large differences in the chemical shifts of both ^{13}C and ^{1}H nuclei which are well removed from the stereotopic aminal center (position 2, 3, 4, 5, 1', 1") are indicative of changes in nuclei environments caused by conformational differences between the two macrocycles (see Table 1 and 2).

The coupling constants seen in the sugar rings are indicative of chair-like conformations. The large values for the diaxial couplings ($J_{2',3'}$ = 10.4 Hz; $J_{3',4'ax}$ = 12.1 Hz; $J_{4'ax,5'}$ = 11.5 Hz; $J_{4",5"}$ = 9.4 Hz) indicate that the six membered sugars adopt the same conformation as that seen in the crystalline conformation of dirithromycin and erythromycin A hydroiodide dihydrate.[6,14] Additionally, the extreme values of the coupling constants in the sugar rings suggest there is little or no conformational equilibrium between the ring-flipped conformations of the two sugars.[20]

TABLE 3: Calculated Coupling Constants ($^3J_{H,H}$) and Corresponding Dihedral Angles for Vicinal Protons of Dirithromycin (**3**) and Epi-Dirithromycin (**4**) in $CDCl_3$.

		DIRITHROMYCIN			EPI-DIRITHROMYCIN		
Vicinal Proton Pairs		^3J (Hz)	Dihedral Angle Φ(°)	Modeled Angle[A] Φ(°)	^3J (Hz)	Dihedral Angle Φ(°)	Modeled Angle[A] Φ(°)
H-2	H-3	1.6	100	100	9.0	155	177.5
H-3	H-4	2.3	-68	-64	< 1.0	-80	-63.1
H-4	H-5	3.5	116	121	7.9	145	132.2
H-8	H-9	9.6	154	159	1.1	81	78.8
H-9	H-10	0.0	90[C]	70	0.9	90	106.6
H-10	H-11	1.1	67	59	1.8	59	61.8
H-1'	H-2'	7.2	180	162	7.2	180	172
H-2'	H-3'	10.3	180[D]	-175	10.2	180[D]	178
H-3'	H-4'ax	12.1	180[D]	-178	12.2	180[D]	-178
H-3'	H-4'eq	3.7	59	63	3.7	59	64
H-4'ax	H-5'	11.5	180[C]	179	---[B]	----	180
H-4'eq	H-5'	1.8	-63	-62	1.8	-63	-62
H-1"	H-2"ax	5.0	-45	-40	4.9	-47	-35
H-1"	H-2"eq	0.0	85[C]	77	0.0	85[C]	82
H-4"	H-5"	9.4	180	180	9.8	180	180

[A]Angles measured on conformations generated by molecular modeling calculations.
[B]Coupling Constants could not be accurately measured.
[C]Corresponds to the calculated dihedral angle given for the smallest available coupling constant.
[D]Corresponds to the calculated dihedral angle given for the largest available coupling constant.

In an effort to access the overall lactone conformation of both **3** and **4**, it was necessary to obtain more information about the spatial proximity of proton pairs in solution. A spatial proximity map[13a] was built from the observed NOEs found in 2D ^1H NOESY and 1D ^1H NOE difference experiments. The NOEs observed for both **3** and **4** are listed in Table 4. The diaxial NOE interactions {[1']5', [2']$4'_{ax}$, [3']5', and [$2"_{ax}$]4"} confirm the conformations of the cladinose and desosamine sugar rings predicted by the coupling constant data. The [1']5, [1']17, [1']5", [5']5" [5"]5, [1"]16 and [1"]3 NOEs define the spatial proximity of the sugars and the lactone ring. The inter-sugar NOEs suggest that the sugars orient such that the α faces are toward each other.

Irradiation of the aminal proton (H-22) of **3** showed enhancements at H-8, H-11, and H-3. The enhancements at H-8 and H-11 are indicative of close proximity to the irradiated proton and suggest that the oxazine may exist in a chair-like conformation with an equatorial alkyl chain at the aminal center (R-configuration). The [22]3 NOE represents a close cross-ring proximity of these protons in solution. Irradiation of the aminal proton (H-22) of **4** showed enhancements at H-9 and H-20. However, these enhancements would not be expected if the oxazine ring maintained a chair-like conformation with the alkyl group in the axial position (aminal with S-configuration). The enhancements at H-9 and H-20 suggest that the proximity of the protons results from an oxazine which exists in a boat-like conformation.

The intra-lactone NOE interactions of [3]8, [3]11, [3]22, [11]8 are observed in **3**, while [4]11, 4[21], and [11]6-OH are the intra-lactone NOE interactions seen in **4**. The NOE data as well as the coupling constant and dihedral angle information suggest that in solution **3** exist in the "folded-in" conformation while **4** exists in a "folded-out" conformation.

TABLE 4: Observed ^1H NOE data from 2D ^1H NOESY experiment (τ_m = 500 ms) for Dirithromycin (**3**) and Epi-Dirithromycin (**4**).

POSITION	DIRITHROMYCIN OBSERVED NOEs	EPI-DIRITHROMYCIN OBSERVED NOEs
2	3, 4, 16, 17	3, 16, 17
3	2, 4, 8, 11, 22, 1"	2, 4, 1"
4	2, 3, 5, 17	3, 6-OH, 11, 17, 21
5	4, 8, 19, 1', 5"	18, 1'
7		8
8	3, 5, 10, 11, 19, 20, 22	7, 19
9	19	19, 20, 22
10	8, 11, 20	11, 20, 21
11	3, 8, 10, 13, 21, 22	4, 10, 13, 21, 6-OH
13	12-OH, 11, 14a, 15, 21	12-OH, 11, 14a, 15, 21
14a	13, 15, 14b, 21	13, 15, 14b, 21
14b	14a, 15, 21	14a, 15, 21
15	13, 14a, 14b	13, 14a, 14b
16	2, 1"	2, 1"
17	1', 2, 4	1', 2, 4
18		5
19	5, 8, 9	8, 9
20	8, 10	9, 10, 22
21	13, 11, 14a, 14b	4, 10, 13, 11, 14a, 14b
22	3, 8, 11	9, 20
1'	5, 17, 3', 5', 5"	5, 17, 3', 5', 5"
2'	4'ax	4'ax
3'	1'	1', 5'
4'ax	2', 4'eq	2', 4'eq
4'eq	4'ax	4'ax
5'	1', 6'	1', 3', 6'
6'	5'	5'
1"	3, 16, 2"ax, 2"eq	3, 16, 2"ax, 2"eq
2"ax	1", 2"eq, 4", 7"	1", 2"eq, 4", 7"
2"eq	1", 2"ax, 7", 8"	1", 2"ax, 7", 8"
4"	2"ax, 6", 7"	2"ax, 6", 7"
5"	5, 1', 6"	1', 6"
6"	4", 5"	4", 5"
7"	2"ax, 2"eq, 4", 8"	2"ax, 2"eq, 4", 8"
8'	2"eq, 7"	2"eq, 7"

A pictorial representation of the conformation of **3** and **4** was obtained through molecular mechanics and semiempirical calculations. Molecular mechanics calculations were performed first with dihedral angle constraints derived from the coupling constant data and distance constraints based upon the observed NOE data. A second minimization was performed releasing all constraints. The geometry of the conformation was then optimized using the AM1 Hamiltonian. The calculated dihedral angles for the energy minimized conformation are in very good agreement with the experimental data (see Table 3). The dirithromycin solution conformation compared very favorably with the crystalline conformation of **3**, while the calculated conformation of **4** compared favorably with the crystalline conformation of erythromycin A hydroiodide dihydrate. The models in Figures 1 and 2 represents the major conformational preferences of **3** and **4**.

Fig. 1: View of the predicted solution conformation of dirithromycin (**3**). Carbons are black, hydrogens are white, oxygens are dotted, and nitrogens are gray. All of the hydrogens except those on the macrocyclic ring have been removed for clarity.

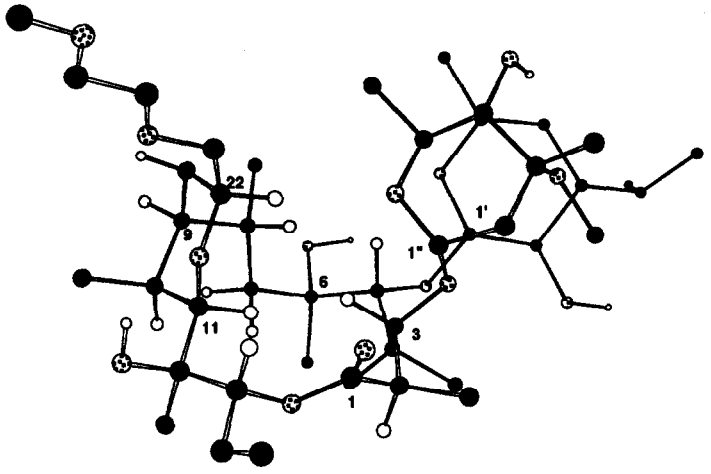
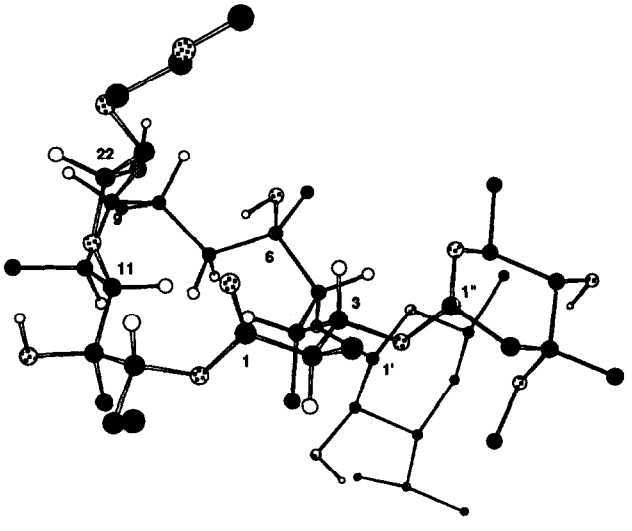


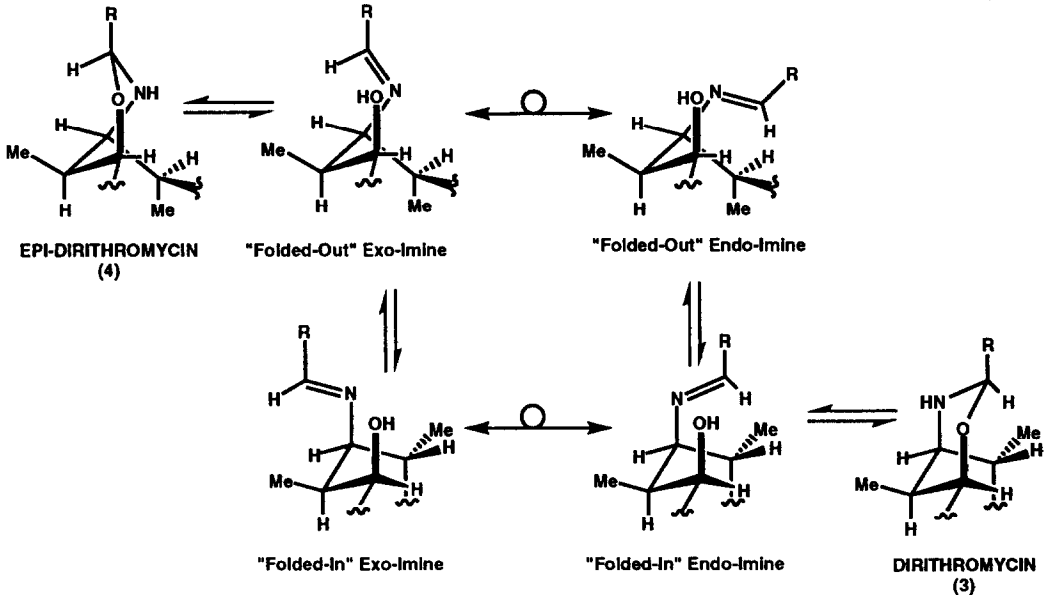
Fig. 2: View of the predicted solution conformation of epi-dirithromycin (**4**). Carbons are black, hydrogens are white, oxygens are dotted, and nitrogens are gray. All of the hydrogens except those on the macrocyclic ring have been removed for clarity.



Kinetic Formation of Epi-Dirithromycin Versus Dirithromycin

Having obtained an understanding of the conformational differences between **3** and **4**, we were interested in rationalizing the stereoselective formation of the oxazine stereocenter. The formation and epimerization of tetrahydro-1,3-oxazines is known to occur via a Schiff base intermediate.[21] Although the Schiff base of **2** and an alkyl aldehyde has never been isolated, the formation of the cyclic aminal must occur via an intermediate imine.[22] It is reasonable to predict that the lactone's conformation in the Schiff base intermediate must be very similar to the product aminal. Hence, **3** must result from the cyclization of the C-11 hydroxyl to the *Re* face of the intermediate imine (endo-imine) with a "folded-in" conformation (Fig. 3). Similarly, **4** results from cyclization on the *Si* face of a Schiff base intermediate (exo-imine) with a "folded-out" conformation (Fig. 3). Examination of molecular models clearly indicates that cyclizations of the "folded-out" endo-imine as well as the "folded-in" exo-imine are unlikely because of the steric and torsional constraints required by these conformations during bond formation. Molecular mechanics calculations suggest a high rotational barrier of the C-9 imine bond from the exo to the endo-imine. The available data suggest that the formation of the transient Schiff base eventually controls the facial selectivity of the oxazine forming cyclization, thus the exo-imine is formed preferentially and immediately cyclizes to give the kinetic product, epi-dirithromycin.[23] Presumably, the preferential formation of the exo-imine results from the avoidance of a large steric interaction between the alkyl chain of the imine and the macrocyclic ring. The understanding of this kinetic preference for exo-Schiff base formation allows us to predict and control the stereochemistry and macrocyclic conformation of oxazines derived from **2**.

Fig. 3: Pictorial representation of the dirithromycin oxazine equilibrium.



CONCLUSION

In conclusion, the unambiguous assignment of the C-22 epimer of dirithromycin has been achieved. In addition, a combination of NMR studies and molecular mechanics calculations have been used to predict the conformation of dirithromycin and epi-dirithromycin in solution. The predicted "folded-in" conformation of **3** is very similar to the crystalline conformation of dirithromycin, while the predicted "folded-out" conformation of **4** is similar to the crystalline conformation of erythromycin A hydroiodide dihydrate. This structural information was used to predict the conformation of the Schiff base intermediates that lead to the formation of the two oxazine isomers. Formation of the "exo-imine" which leads to **4** proved to be kinetically preferred. The ability to predict and control the conformation of erythromycylamine derivatives has both chemical and medicinal utility. This work provides some rationale for the stereoselective and conformationally biased formation of aminals derived from (9S)-erythromycylamine.

EXPERIMENTAL

All NMR spectra were recorded at 0 °C on a Bruker AC300 spectrometer equipped with a 5 mm, $^{1}H/^{13}C$ dual probe and operating at frequencies of 300.13 and 75.47 MHz, respectively. All samples were prepared using *ca.* 300 mg/ml in $CDCl_3$, unless otherwise specified. All spectra are referenced to an external TMS standard.

The ^{1}H NMR spectra were acquired with a spectral width of 4065 Hz using 32K data points. The ^{13}C NMR spectra were acquired using a 64K data set with a spectral width of 18518 Hz. The DEPT spectra (45°, 90°, and 135°)[24] were acquired under the same spectral parameters and using a polarization transfer delay corresponding to a $^{1}J_{CH}$ value of 145 Hz.

The 2D $^{1}H,^{1}H$ COSY NMR spectra[25] were acquired using 90° pulses (8 μs) on a spectral width of 1501 Hz using 2048 data points in the f_2 domain. Each of the 256 FIDs was comprised of 32 scans and 2 dummy scans with a relaxation delay of 1 s between scans. The f_1 domain was zero-filled from 256 out to 1024 points. Prior to Fourier transformation, the data was multiplied by a sine-bell window function in both f_2 and f_1.

A 2D $^{13}C,^{1}H$ COSY NMR experiment[26] was run using spectral widths of 749 Hz and 7936 Hz with 512 and 4096 data points, respectively. The 90° pulse lengths were 26.5 and 5.9 μs, respectively. A polarization transfer delay was used corresponding to a $^{1}J_{CH}$ value of 145 Hz. Each of the 128 FIDs was acquired with 256 scans and 2 dummy scans and a relaxation delay of 1 s. The data in f_1 was zero-filled from 128 to 512 points prior to Fourier transform. A Lorentzian window function was used in f_2 with a 2 Hz line-broadening and a shifted sine-bell window function was used in f_1.

A 2D $^{13}C,^{1}H$ Long-Range COSY NMR experiment, sometimes referred to as the FUCOUP experiment,[26] maps all heteronuclear J couplings seen between carbon and proton nuclei which are 1, 2, 3, and more bonds apart. Thus, connectivity assignments were made by correlating the long range coupling of protons with previously assigned carbon resonances. The spectra were acquired with spectral widths of 1593 Hz and 16667 Hz into 512 and 2048 points, respectively. Each of the 128 FIDs was acquired with 1400 scans and 2 dummy scans with a 1s relaxation delay between scans. The data in f_1 was zero-filled from 128 out to 512 points. Prior to transformation, each domain was multiplied by a Gaussian window function using a line-broadening factor of -1.5 Hz and a 0.3 Gaussian factor.

The 2D homonuclear ^{1}H J-resolved NMR data was acquired using the Hahn spin-echo.[11] The acquisition ran with a spectral width of 1524 Hz using 4096 data points in f_2 and a spectral width of 48 Hz using 128 data points in f_1. The data in f_1 was zero-filled out to 1024 points. A sine-bell window function was used in both f_1 and f_2 prior to Fourier transformation.

The 2D phase-sensitive ^{1}H NOESY NMR experiment was acquired using the TPPI method on a spectral width of 1667 Hz using 2048 data points.[27] Samples prepared for NOE studies contained *ca.* 50 mg/ml of

sample in $CDCl_3$. Each of 512 FIDs were acquired with 16 scans and 2 dummy scans and utilized a 3 s relaxation delay between scans. The mixing time was 500 ms with a 4% random variation.

The ^1H NOE difference spectra were acquired using one off-resonance irradiation and five on-resonance irradiations per experiment. Automatic cycling through the frequency list was used, taking 8 scans and 2 dummy scans for each frequency per cycle. The total delay for relaxation was 5.3 s. The total number of scans for each frequency was 1200. The duration for irradiation was 500 ms. Each FID collected was 16K data points, but was zero-filled out to 64K before Fourier transformation and subsequent subtraction.

Molecular mechanics calculations were performed using the Quanta/CHARMm molecular modeling programs.[28] The starting oxazine structure was generated from the X-ray structure of either (9S)-9-N, 11-O-[2-(2-methoxyethoxy)ethylidene]erythromycylamine A (Dirithromycin)[6] or 9-[O-(2,5-dioxahexyloxime]-erythromycin A (Roxithromycin),[29] which were obtained through interface with the Cambridge Crystallographic Database. Minimizations were performed using the Adopted-Basis Newton Raphson method with dihedral angle constraints generated from NMR coupling constant data and distance constraints determined by NOE data. The constraint force constant was calculated using *SCALE* = 50 - 200 (Distance Constraint Files) and *Weight* = 50 - 200 (Dihedral Angle Constraint Files) at 300 °K. A cutoff distance of 3Å was used in calculating the distance constraint force constants. Semiempirical geometry optimizations were performed in MOPAC 5.0 using AM1 parameters.

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