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CONFORMATIONAL ANALYSIS OF TRICYCLIC KETOLIDE TE-802 AND ITS ANALOGUES

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Abstract – The conformations of tricyclic ketolide TE-802 (**1**) and its analogue were determined by crystallographic analysis and NMR spectroscopy. The 3-carbonyl function of **1** took an '*exo*' orientation against the lactone ring, and the orientation of the amino sugar at the 5-position differed from that in clarithromycin (CAM:**2**). On the other hand, di- and tricyclic ketolides and a tricyclic macrolide were found to exist in 'semi-fold-out (4H-downward)' conformation in deuteriochloroform. The conformation of **1** in deuteriochloroform was closely similar to that in D_2O. The newly formed tetrahydrodiazepine ring existed in chair form or boat form, governed by the presence of a substituent and its position.

1. INTRODUCTION

Erythromycin, one of the most important macrolide antibiotics, has been used for almost a half-century against many infectious diseases, especially respiratory tract infections caused by Gram-positive bacteria and *Mycoplasma* bacteria, *Mycoplasma*, *Clamydia* and *Legionella*. A major problem of erythromycin is its acid instability,[1] which results in poor bioavailability and gastrointestinal side effects.[2] To improve instability in acid, so-called second-generation macrolides such as clarithromycin (**2**) ,[3] azithromycin (**3**)[4] and roxithromycin[5] were introduced in the late 1980s. However, due to the recent emergence of macrolide resistance, new agents with activity against resistant bacteria are needed. In 1995, our group[6] and Aventis Pharma[7] introduced a new series of macrolides designated the 'ketolides' at the same scientific conference. Ketolides exhibited great *in vitro* and *in vivo* activity against several types of macrolide-resistant organisms. A common structural feature of ketolides is possession keto function at the 3-position instead of the original cladinose sugar, which had hitherto been considered essential for

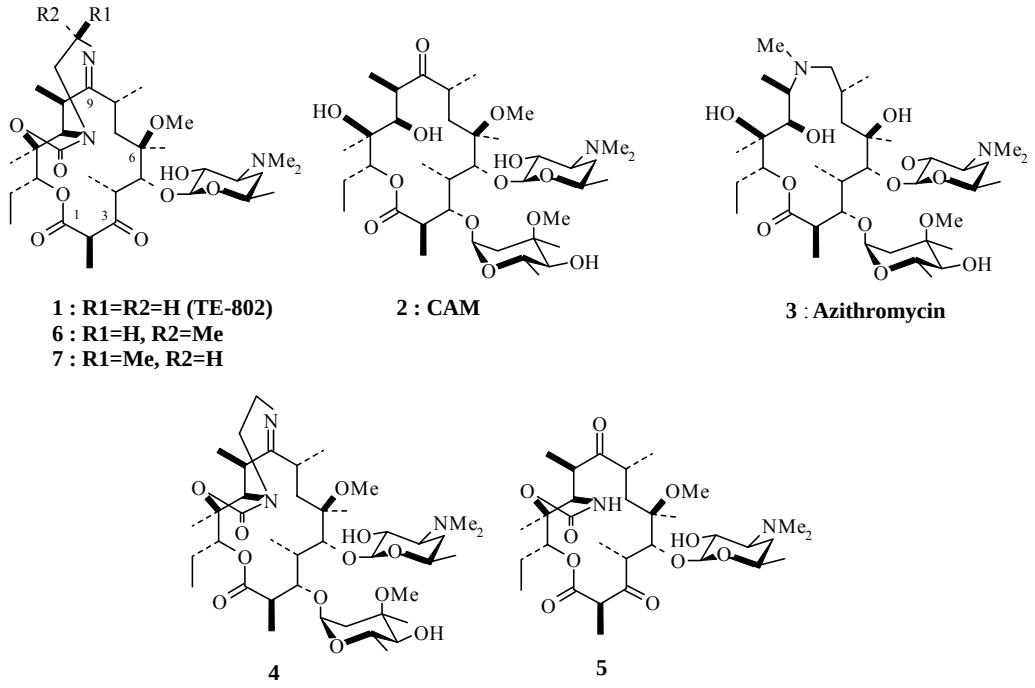


Figure 1

antibacterial activity. In addition, an original prominent characteristic of our compounds was formation of a unique tricyclic structure in the aglycon moiety. Due to their unique skeleton and excellent antibacterial activity,[8] the tricyclic ketolides have attracted much attention. After our publication, many researchers have used this skeleton as a scaffold for further investigation in the development of new ketolide antibiotics.[9] Therefore, it is very significant to characterize the physicochemical properties of tricyclic ketolides. In this paper, we report global conformations of TE-802 and its analogues, tricyclic macrolides and ketolides, in solid and solution phases determined using crystallographic analysis and NMR spectroscopic methods.

2. RESULTS AND DISCUSSION

2.1 Crystallographic analysis

2.1.1 Torsion angles

In order to discuss the conformation of tricyclic ketolides and other macrolides in solid phase, we determined molecular structures of TE-802 (**1**), **4** and **5**[11] by X-Ray crystallographic analysis. Reported X-Ray crystallographic data for **2**[10] and **6**[8a] were also used for detailed analysis. The conformation of aglycon was found to be determined by three factors: 1) formation of a diazepine ring, 2) conformation of the diazepine ring (9-N, 11-N-ethano bridge), 3) possession of a 3-carbonyl or original cladinose. Crystal data for **1**, **4** and **5** are summarized in the EXPERIMENTAL.

Comparison of the original macrolide (**2**) with **4**, which featured introduction of a tricyclic structure into its aglycon moiety, revealed great similarity in superposition of the aglycon (Figure 2). The torsion angles of the lactone ring in **2** and **4** were also similar (Table 1). Although there was a slight difference in the C10-C11-C12-C13 region (**2** : 169º, **4** : 157º, Δ = 12º), the torsion angles of aglycon in **4** agreed well with those of **2**. These findigs indicate that formation of a tricyclic structure alone does not have a large impact on change of the aglycon's conformation. The orientation of the desosamine sugar is altered in **4** compared with that in **2** without change of aglycon moiety. The desosamine in **4** was slightly closer to the cladinose linking at the 3-position.

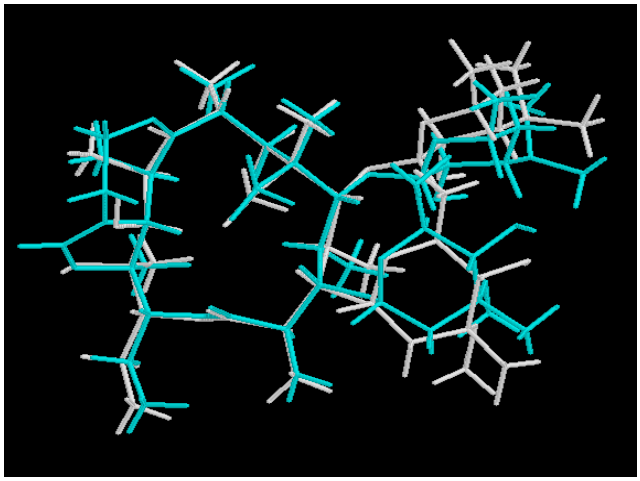


Figure 2. Superimposition of **2** (white) and **4** (cyan) crystal structures.

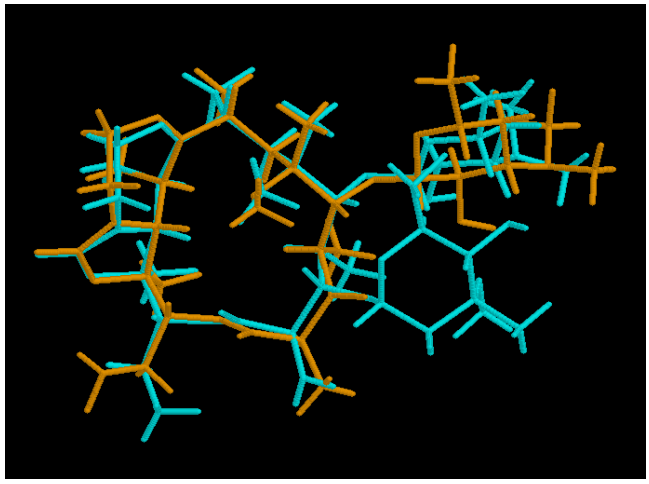


Figure 3. Superimposition of **1**(orange) and **4** (cyan) crystal structures.

Table 1. Torsion angles of lactone ring (deg)

	2 : CAM[10)]	**1**	**4**	**5**	**6**
C13-O14-C1-C2	175	173	168	176	168
O14-C1-C2-C3	124	102	115	117	118
C1-C2-C3-C4	-92	-69	-80	-104	-100
C2-C3-C4-C5	157	155	164	157	153
C3-C4-C5-C6	-86	-126	-97	-86	-82
C4-C5-C6-C7	-74	-61	-72	-72	-70
C5-C6-C7-C8	-177	179	179	-177	-172
C6-C7-C8-C9	-74	-74	-74	-66	-66
C7-C8-C9-C10	-67	-56	-63	-69	-75
C8-C9-C10-C11	117	121	122	133	137
C9-C10-C11-C12	-172	-172	-173	-170	-165
C10-C11-C12-C13	169	150	157	150	146
C11-C12-C13-O14	-71	-84	-68	-74	-70
C12-C13-O14-C1	114	150	118	129	125

Next, we compared **4** with **1**, which was a 3-carbonyl derivative of **4** (Figure 3). The torsion angles of C3-C4-C5-C6 and C12-C13-O14-C1 regions in **1** differed from those in **4**. Interestingly, the desosamine sugar moved from the original position to the upper side of the lactone ring in ketolide (**1**). A similar phenomenon was observed in ketolide (**5**). It is commonly accepted that 2'-OH and 3'-N$(CH_3)_2$ functions play important roles in binding to ribosomes, and that the orientations and angles of these two functions to the aglycon are particularly significant in this respect. This alteration is thus essential to the excellent activity of ketolides, as opposed to typical macrolides. The torsion angles of aglycon in **1** differed markedly from those of **2** in the C3-C4-C5-C6 and C12-C13-O14-C1 regions (Δ=40º and 36º, respectively). Both the 1- and 3-carbonyl groups took the '*exo*' orientation against the lactone ring. This peculiar finding was observed only in **1**, which has both a tricyclic aglycon and 3-carbonyl function. Actually, the corresponding torsion angles of aglycons in **4**, a tricyclic macrolide with a cladinose sugar, and **5**, ketolide with a dicyclic aglycon, were similar to those of **2** (**4** : Δ=11º and 4º, **5** : Δ=0º and 15º) in corresponding regions. Contrary to our expectation, the torsion angles of C3-C4-C5-C6 and C12-C13-O14-C1 regions in **6**, which has both a tricyclic aglycon and 3-carbonyl function, were similar not to those of **1** but to those of **2** (Δ=4º and 11º). This finding was due to ring conversion of the tetrahydrodiazepine moiety in **6**. Compound (**6**) took a twisted conformation on the 9-N, 11-N-ethano bridge moiety, unlike **1**, on X-Ray crystallographic analysis (Figure 4). It appeared that the methyl group on the 9-N, 11-N-ethano bridge moiety preferred an equatorial conformation in the diazepine ring, and the 9-N, 11-N-ethano bridge therefore had to twist. We don't have positive reason why both 1- and 3-carbonyl groups of only **1** take '*exo*' orientation against the lactone ring so far. But it is certain that the possession of 3-keto function and non-twisted diazepine ring at the same time is indispensable to take such a particular conformation by crystallographic analysis of **2**, **4**, **5** and **6**.

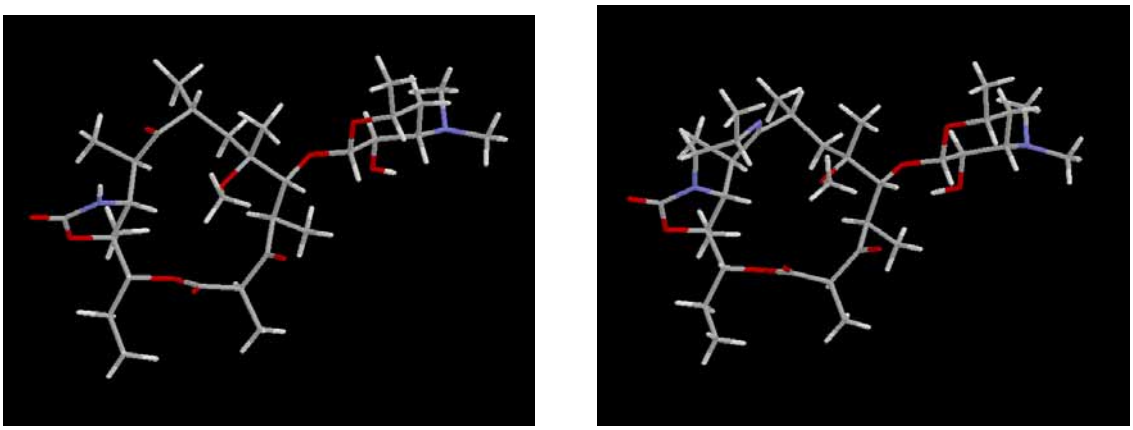


Figure 4. X-Ray crystal structures of compounds (**5**) and (**6**).

2.1.2 Distances between principal protons

Steinmetz *et al*. carried out a detailed analysis of the conformation of **2** in aqueous solution, and concluded that **2** exists as a fold-out conformer in aqueous solution.[12] Barber *et al*. calculated distances between principal protons in the aglycon ring for **2** in fold-out and fold-in conformations, and reported that NOEs between H4-H11, H5-6Me, H8-6Me and 2Me-14Me should be observed in the fold-out conformation.[13] In their study, distances between the principal protons in the aglycon ring for **1, 2, 4, 5** and **6** were investigated and the results are summarized in Table 2. Distances of H5-6Me and H8-6Me in **2** were both 2.8 Å. Corresponding distances in **1**, **4**, **5** and **6** ranged from 2.8 Å through 3.1 Å. In these four compounds, distances of H5-6Me and H8-6Me were similar to those in **2** in the fold-out

Table 2. Internuclear distances in lactone ring (Å)

	2*						
	cryatal*	calculation**		**1**	**4**	**5**	**6**
Contact		fold-out	fold-in				
H3-H8	5.5	5.8	2.1	-	5.7	-	-
H3-H11	3.7	3.8	2.5	-	3.6	-	-
H4-H11	3.1	2.4	3.3	2.9	2.9	3.6	3.8
H4-6Me	4.5	4.6	2.1	4.4	4.4	4.6	4.5
H5-6Me	2.8	2.4	3.8	3.1	2.9	3.0	2.9
H8-H11	4.1	4.2	2.3	4.1	4.4	4.2	4.3
H8-6Me	2.8	2.2	4.5	2.9	3.0	2.8	2.8
2Me-14Me	4.5	2.6	3.3	6.1	4.4	4.6	4.5
2Me-4Me	4.2	4.0	2.3	4.5	4.3	4.0	4.0

* Reference 10.
** Reference 13.

conformation. However, the distance of H4-H11 in **2** was 3.1 Å, and **1** and **4** exhibited values very similar (both 2.9 Å) to that of **2**. The corresponding values in **5** and **6** were 3.6 Å and 3.8 Å, respectively, and far larger than even the calculated value for the fold-in conformation in **2** (3.3 Å). With removal of cladinose at the 3-position, the desosamine sugar moved to the upper side of the lactone ring in **5** and **6**, while H4 was dragged to the back of the lactone ring. Thus the distances of H4-H11 in **5** and **6** became large. On the other hand, **1** maintained the distance of H4-H11 (2.9 Å) by maintaining an '*exo*' orientation of both 1- and 3-carbonyl groups against the lactone ring. Girault *et al*. reported that RU-004 (a ketolide antibiotic) had 'up' positions for the three carbonyl groups (1-CO, 3-CO and 9-CO) in solution as the major conformation, and that distance between H4-H11 was 4.3 Å.[14] This result coincided with ours for ketolides (**5**), a dicyclic ketolide, and **6**, with a twisted tetrahydrodiazepine ring.

Actual 2Me-14Me distances in **1**, **4**, **5** and **6**, including **2**, differed from the calculated value for **2**.

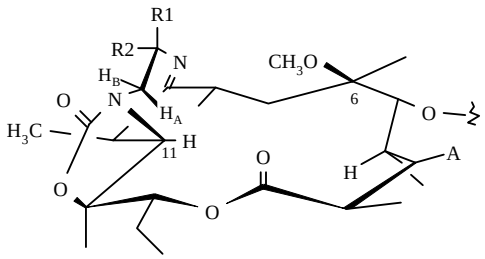
However the calculated value for 2Me-14Me in **2** existing in fold-out conformation was 2.6 Å, corresponding to the actual measured values in **1**, **2**, **4**, **5** and **6** of 6.1 Å, 4.5 Å, 4.4 Å, 4.6 Å and 4.5 Å, respectively. These observed values were far larger than the calculated value for the fold-in conformation of **2** (3.3 Å), while NOE between 2Me-14Me was observed in every compound in the NMR spectroscopic study. These results suggest that the 2Me and 14Me groups of **1**, **4**, **5** and **6** are able to be close to each other in solution.

In conclusion, although the conformation of tricyclic macrolide (**4**) was very similar to that of **2**, ketolide compounds (**5**) and (**6**) took the 3-carbonyl 'up' position as well as RU-004. On the other hand, **1** took the characteristic conformation with 1- and 3-carbonyl '*exo*' orientation against the lactone ring, unlike **2**, **4**, **5** and **6**.

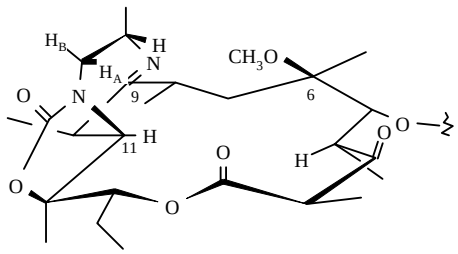
2.2 NMR Spectroscopic Study

2.2.1 Tetrahydrodiazepine ring

The chemical shifts of H_B for **1**, **4** and **7** were similar (3.99, 3.99 and 3.85 ppm, respectively), and were fairly large compared with those of H_A for **1**, **4** and **7** (2.95, 3.01 and 2.67 ppm, respectively, see Table 3 and reference 8a). This difference appeared to be due to the anisotropic effect of the carbonyl group on 11,12-cyclic carbamate. As shown in figure 5, in the case of **1**, **4** and **7**, H_B was located in parallel to the carbonyl bond on 11,12-cyclic carbamate, and H_B signals thus appeared at lower field than the H_A signals. Chemical shifts of H_A and H_B for **1** in D_2O were 3.25 and 4.16 ppm, respectively, indicating that the conformation of the tetrahydrodiazepine ring for **1** in $CDCl_3$ is maintained in D_2O. On the other hand, compound (**6**) exists in the twisted form on the 9-N, 11-N-ethano bridge moiety in solution, as in solid state.



1 : R1=R2=H, A= =O
4 : R1=R2=H, A=-O-cladinose
7 : R1=CH_3, R2=H



6

Figure 5

By taking the twisted form, the carbonyl bond on the 11,12-cyclic carbamate in **6** stands exactly in between H_A and H_B, so the chemical shifts of H_A and H_B were very similar (3.38 and 3.47 ppm,

respectively) due to the approximately equal the anisotropic effects of the carbonyl group on them. The chemical shifts of H_A and H_B for **6** were exactly moderate in corresponding values for **1**, **4** and **7**.

Table 3. Proton chemical shift and coupling constants for **1** at 25°C

	in $CDCl_3$		in D_2O	
	ppm (δ)	^3J(Hz)	ppm (δ)	^3J(Hz)
H-2	3.82	6.7 (2-2Me)	--	
H-4	3.08	8.5 (4-5)	3.23	6.1 (4-5)
H-5	4.21		4.39	
H-7_A	1.50	11.6 (7_A-8)	1.88	2.1 (7_A-8)
H-7_B	1.71	2.4 (7_B-8)	2.05	11.6 (7_B-8)
H-8	2.70	7.0 (8-8Me)	3.09	6.1 (8-8Me)
H-10	2.73	1.5 (10-11)	3.42	
H-11	3.73		3.83	
H-13	4.95	10.6 (13-14_A)	5.03	10.7 (13-14_A)
		2.4 (13-14_B)		2.3 (13-14_B)
H-14_A	1.55	7.7 (14_A-14Me)	1.73	7.3 (14_A-14Me)
H-14_B	1.91	7.7 (14_B-14Me)	1.85	7.3 (14_B-14Me)
2-Me	1.39		1.35	
4-Me	1.29	7.0 (4-4Me)	1.31	6.9 (4-4Me)
6-Me	1.36		1.34	
8-Me	1.05		1.30	
10-Me	1.22	7.0 (10-10Me)	1.42	6.9 (10-10Me)
12-Me	1.48		1.68	
14-Me	0.86		0.87	
9-NCH_A(eq.)	3.78	4.9 (9NCH_A-11NCH_A)	3.90	2.1 (9NCH_A-11NCH_A)
		2.8 (9NCH_A-11NCH_B)		2.4 (9NCH_A-11NCH_B)
9-NCH_B(ax.)	3.78	9.7 (9NCH_B-11NCH_A)	4.14	11.3 (9NCH_B-11NCH_A)
		2.8 (9NCH_B-11NCH_B)		2.1 (9NCH_B-11NCH_B)
11-NCH_A	2.95		3.25	
11-NCH_B	3.99		4.16	
6-OMe	2.74		2.81	
H-1'	4.30	7.3 (1'-2')	4.49	6.9 (1'-2')
H-2'	3.19	10.0 (2'-3')	3.54	6.9 (2'-3')
H-3'	2.44	12.2 (3'-4'$_A$)	3.52	12.2 (3'-4'$_A$)
		3.7 (3'-4'$_B$)		4.3 (3'-4'$_B$)
H-4'$_A$	1.23	10.9 (4'$_A$-5')	1.60	12.1 (4'$_A$-5')
H-4'$_B$	1.67	2.1 (4'$_B$-5')	2.17	1.5 (4'$_B$-5')
H-5'	3.54	6.0 (5'-5'Me)	3.85	6.1 (5'-5'Me)
5'-Me	1.24		1.35	
3'-NMe_2	2.26		2.87	
2'-OH	3.49		--	

Table 4. Coupling constants for **4, 5, 6** and **7** (aglycon ring)

	Coupling constants ^3J(Hz)			
	4	**5**	**6**	**7**
H-2	8.8 (2-3)	6.7 (2-2Me)	6.7 (2-2Me)	6.7 (2-2Me)
H-3	<1 (3-4)			
H-4	7.6 (4-5)	7.9 (4-5)	9.2 (4-5)	8.5 (4-5)
H-5				
H-7_A	9.8 (7_A-8)	12.2 (7_A-8)	12.2 (7_A-8)	14.0 (7_A-8)
H-7_B	3.3 (7_B-8)	2.4 (7_B-8)	2.4 (7_B-8)	1.8 (7_B-8)
H-8	7.0 (8-8Me)	6.4 (8-8Me)	6.7 (8-8Me)	7.3 (8-8Me)
H-10	1.5 (10-11)	<1 (10-11)	6.7 (10-10Me)	6.7 (10-10Me)
H-11				
H-13	11.0 (13-14_A)	10.6 (13-14_A)	10.4 (13-14_A)	10.4 (13-14_A)
	2.1 (13-14_B)	2.5 (13-14_B)	2.4 (13-14_B)	2.5 (13-14_B)
H-14_A	7.3 (14_A-14Me)	7.6 (14_A-14Me)	7.3 (14_A-14Me)	7.3 (14_A-14Me)
H-14_B	7.3 (14_B-14Me)	7.6 (14_B-14Me)	7.3 (14_B-14Me)	7.3 (14_B-14Me)
2-Me	7.3 (2-2Me)			
4-Me	7.6 (4-4Me)	7.6 (4-4Me)	7.3 (4-4Me)	7.3 (4-4Me)
6-Me				
8-Me				
10-Me	7.0 (10-10Me)	6.4 (10-10Me)		
12-Me				
14-Me				
9-NCH_A	3.1 (9NCH_A-11NCH_A)		3.1 (9NCH-11NCH_A)	6.7 (9NCH-9NCMe)
	2.7 (9NCH_A-11NCH_B)		6.7 (9NCH-11NCH_B)	11.0 (9NCH-11NCH_A)
			7.3 (9NCH-9NCMe)	2.5 (9NCH-11NCH_B)
9-NCH_B(ax.)	11.6 (9NCH_B-11NCH_A)			
	2.7 (9NCH_B-11NCH_B)			

Kasprzyk and Kolinski studied the conformation of 2,3,6,7-1H-1,4-diazepine in solution, and reported that it could normally exist in chair conformation.[15] The coupling constants of 9NCH-11NCH_B and 9NCH-11NCH_A for **7** in $CDCl_3$ were 2.5 Hz and 11.0 Hz, respectively, and agreed with the results of calculation for the chair form of 2,3,6,7-1H-1,4-diazepine obtained by Kasprzyk *et al*. The coupling constants of **1** (9NCH_B-11NCH_B : 2.8 Hz, 9NCH_B-11NCH_A : 9.7 Hz) and **4** (9NCH_B-11NCH_B : 2.7 Hz, 9NCH_B-11NCH_A : 11.6 Hz) suggested that these compounds also exist in chair form. Moreover, **1** exists in the same chair conformation in D_2O (9NCH_B-11NCH_B : 2.1 Hz, 9NCH_B-11NCH_A : 11.3 Hz) as in $CDCl_3$. On the other hand, **6** appeared to be in boat conformation as a result of taking a twisted form on the 9-N, 11-N-ethano bridge moiety. The coupling constants of 9NCH-11NCH_B and 9NCH-11NCH_A for **6** in $CDCl_3$ were 6.7 Hz and 3.1 Hz, respectively, unlike those calculated for the boat form (10.0 Hz and 8.5 Hz). Regrettably, Kasprzyk *et al*. did not measure the coupling constants for the boat form of 2,3,6,7-1H-1,4-diazepine spectral. We established that **6** existed in a boat form in solid state, and ^1H

NOE data from the 2D ^{1}H NOESY experiment suggested that **6** existed in a boat form in solution as well.[8a] In conclusion, each methyl group on the 9-N, 11-N-ethano bridge moiety in **6** and **7** is in the energetically favorable equatorial conformation. As a result, **1**, **4** and **7** exist in chair form, whereas **6** exists in boat form in solution.

2.2.2 Conformation of Aglycon

Everett and Tyler demonstrated that erythromycin A exists predominantly (over 90%) in the fold-out conformation.[16] In contrast, it is known that dirithromycin,[17] which has an oxazine ring in its aglycon moiety, and **3**, a 15-membered azalide, exist in fold-in conformation in solution.[18] We therefore investigated the conformation of the aglycon portion of tricyclic macrolides by NMR spectroscopy. As mentioned above, NOE interaction is observed between H4-H11, H5-6Me, H8-6Me and 2Me-14Me when the compound exists in fold-out conformation in solution. Moreover, NOE interaction is observed between H3-H8, H3-H11, H4-6Me, H8-H11 and 2Me-4Me when the compound exists in fold-in conformation. ^{1}H NOE data from the 2D ^{1}H NOESY experiment for **1, 4, 5, 6** and **7** in $CDCl_3$ and ^{1}H ROE data from the 2D ^{1}H ROESY experiment for **1** in D_2O are summarized in Table 5.

Table 5. NOEs for **1**, **4**, **5**, **6** and **7**

Compound No.	Fold-out				Fold-in				
	H4-H11	H5-6Me	H8-6Me	2Me-14Me	H3-H8	H3-H11	H4-6Me	H8-H11	2Me-4Me
1	no	ob	ob	ob	--	--	no	no	ob
1*	no	ob	ob	ob	--	--	no	no	**
4	no	ob	ob	ob	no	no	no	no	**
5	no	ob	ob	ob	--	--	no	no	ob
6	no	ob	ob	ob	--	--	no	no	ob
7	no	ob	ob	ob	--	--	no	no	ob

* ROESY data in D_2O.
** NOE and ROE crosspeak could not be accurately determined by noise.
no : not observed.
ob : observed.

We performed ROESY experiment instead of NOESY for **1** in D_2O, because the latter did not yield accurate measurement of NOE interaction for **1** in water. NOE interactions between H3-H8 and H3-H11 in ketolide are absent from the beginning. The intra-lactone NOE interactions of H5-6Me, H8-6Me and 2Me-14Me were observed in all investigated compounds, but were not observed between H4-H11. Furthermore, NOE interaction of 2Me-4Me in **1, 5, 6** and **7**, which should be observed in fold-in conformation, was observed. In the case of **4**, the interaction of 2Me-4Me could not be accurately confirmed. The H4-H11 distance in **1** was comparatively small (2.9 Å) in the X-Ray crystallographic

study, but NOE interaction was not observed, indicating that **1** takes the 3-CO 'up' conformation, just like other ketolides in solution. Thus, H4 moved to the back of the lactone ring in all investigated compounds including **1** in solution. As a result, the NOE interaction of 2Me-4Me was observed but that of H4-H11 was not in **1, 4, 5, 6** and **7**. Tricyclic macrolide (**4**), which has an original cladinose sugar at the 3-position, exhibited great similarity to **2** in superposition of the aglycon moiety, but NOE interaction of H4-H11 was not observed. The H4-H11 distance in **4** (2.9 Å) was identical to that in **2**, but **4** took a conformation in solution different from that in crystal. The fold-out conformation is characterized by close cross-ring proximity of C-4 and C-11, and relatively large coupling constant values for $J_{2,3}$ (10 Hz) and $J_{4,5}$ (8 Hz) .[19] The fold-in conformation is characterized by close cross-ring proximity of C-3 and C-11, and relatively small coupling constant values for $J_{2,3}$ (2 Hz) and $J_{4,5}$ (3 Hz). According to their report, 5 investigated compounds preferred the fold-out conformation. The coupling constant values of $J_{4,5}$ in **1, 4, 5, 6** and **7** were 8.5, 7.6, 7.9, 9.2 and 8.5Hz, respectively, while that in **1** in D_2O was 6.1 Hz. In conclusion, these findings revealed that tricyclic macrolides existed in so-called 'semi-fold-out' conformation in solution.

2.2.3 Effects of pD and Temperature

The ^{1}H NMR chemical shift assignments as well as coupling constant values for **1** in $CDCl_3$ and D_2O are listed in Table 3. For the D_2O solution, pD was adjusted to 6.0 with the addition of 20% DCl solution. Coupling constants for **1** in D_2O closely matched those in $CDCl_3$, indicating that **1** existed in approximately similar conformation in $CDCl_3$ and D_2O. On the other hand, the coupling constant values of H_{7A}-H_8 and H_{7B}-H_8 were reversed in each solvent. Corresponding values in $CDCl_3$ for **1** were 11.6 Hz and 2.4 Hz, but those in D_2O were 2.1 Hz and 11.6 Hz. It appeared that this reversal was not due to change of conformation but to protonation by DCl at the 9-imino function, since no marked change in coupling constant values around the 7 and 8 positions was observed. The effect of protonation due to the addition of DCl was observed as chemical shifts of some protons. Proton signals of $9NCH_A$ and $9NCH_B$ in D_2O were observed at 3.90 ppm and 4.14 ppm separately, but could not be separated in $CDCl_3$ (3.78 ppm). Moreover, chemical shifts of H3' and 3'-$N(CH_3)_2$ were shifted downfield from 2.44 ppm and 2.26 ppm in $CDCl_3$ to 3.52 ppm and 2.87 ppm in D_2O by protonation of the 3'-dimethylamino group. The proton at the 2-position is caught between the lactone and ketone functions. It is therefore acidic enough to change to the deuterium in D_2O. H2 signal could therefore not be observed in D_2O. Barber *et al*. measured $J_{H2,H3}$ for azithromycin and erythromycin A over range 20-80 ºC in aqueous solution, and reported $J_{H2,H3}$ values for both drugs changed by less than 0.5 Hz with temperature[13]. In order to examine the effect of temperature on conformational change in tricyclic ketolide, we also measured

proton NMR of **1** at 26 ºC and 45 ºC in $CDCl_3$. Consequently, chemical shifts and coupling constants of **1** at 26 ºC and 45 ºC were similar (Figure 6).

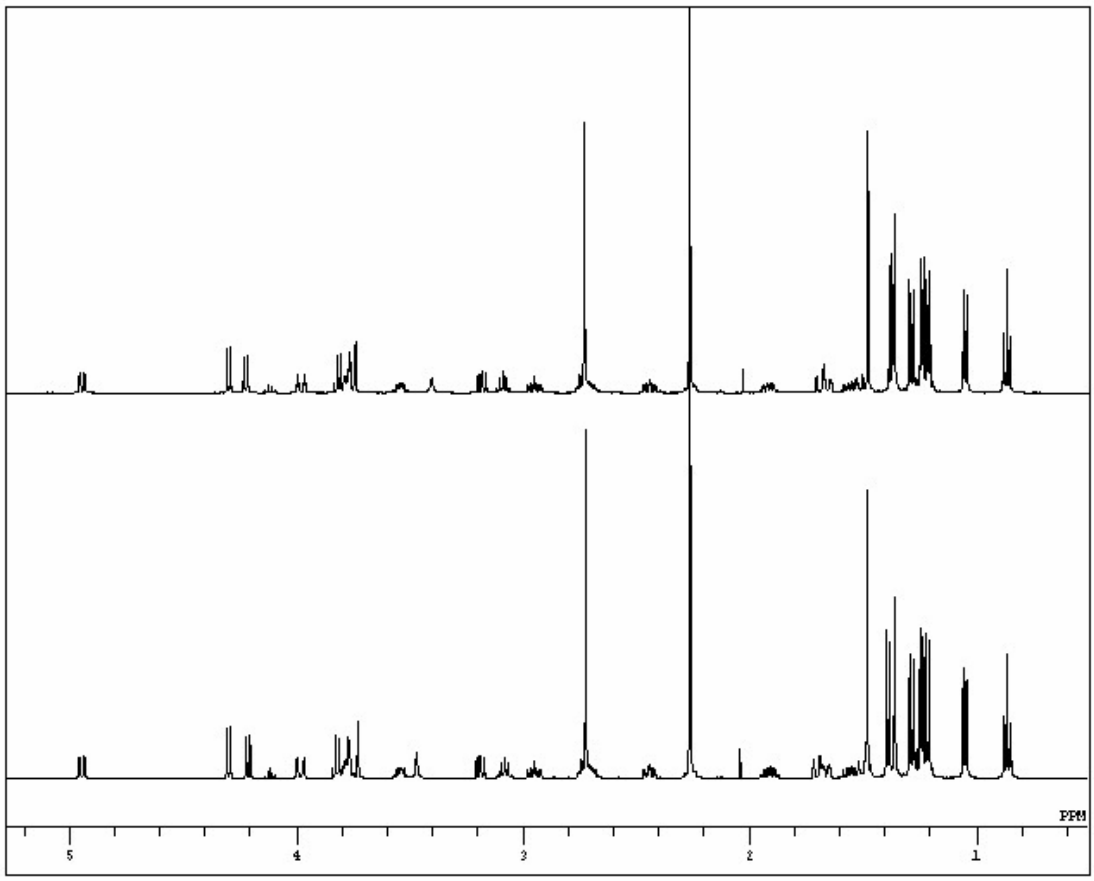


Figure 6 ^{1}H NMR spectra for**1** in $CDCl_3$ at 45℃ (upper) and 26℃ (lower).

3. Conclusion

The conformation of tricyclic ketolides is determined by three factors: 1) formation of a diazepine ring, 2) the conformation of the diazepine ring (9-N, 11-N-ethano bridge moiety), 3) possession of a 3-keto or original cladinose, as determined by the X-Ray crystallographic analysis. The common conformational feature of ketolides is the orientation of the desosamine attached to the 5-position. Ketolides (**5**) and (**6**) take the 3-carbonyl 'up' conformation, while only **1** is took the characteristic conformation with both 1- and 3-carbonyl groups in 'exo' orientation against the lactone ring. Tricyclic macrolides (**1**, **4**, **6** and **7**) and ketolide (**5**) were found to exist in 'semi-fold-out (4H-downward)' conformation in solution, and the conformation of **1** was unaffected by type of solvent and temperature. The exellent *in vitro* and *in vivo* efficacies of tricyclic ketolides may depend on the orientation of the desosamine sugar attached to the 5-position and the rigitity of their conformations.

4. EXPERIMENTAL

4.1 General

Compounds (**1**, **4**, **5**, **6** and **7**) were prepared at Taisho Pharmaceutical Co., Ltd.

4.2 X-Ray crystalographic analysis

X-Ray diffractions were measured on a Mac Science/Bruker axs MXC18 four-circle automated diffractometer with monochromated Cu-Kα (λ=1.54178) radiation at 288 K. Structures were determined by direct methods using SHELXS86. The non-hydrogen atoms were refined anisotropically, while only coordinates of hydrogen atoms were refined. All calculations were performed using the CRYSTAN-G crystallographic software package from Mac Science/Bruker axs. Crystal data[20] for **1**, **4** and **5** are summarized in Table 6.

Table 6. Crystal data for **1**, **4** and **5**

	1	**4**	**5**
Chemical formula	$C_{33}H_{55}N_3O_9$	$C_{41}H_{71}N_3O_{12}\cdot C_3H_6O$	$C_{31}H_{52}N_2O_{10}$
Formula weight	637.82	856.11	612.76
Crystal size / mm	0.55x0.55x0.50	0.35x0.40x0.30	0.50x0.55x0.50
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$ (#19)	$P2_12_12_1$ (#19)	$P2_12_12_1$ (#19)
a	15.052 (4) Å	20.404 (3) Å	12.260 (2) Å
b	16.632 (3) Å	25.587 (4) Å	25.040 (5) Å
c	13.590 (2) Å	9.021 (2) Å	10.956 (3) Å
V	3402 (1) $Å^3$	4709 (1) $Å^3$	3363 (1) $Å^3$
Z	4	4	4
Dc / ($g\cdot cm^{-3}$)	1.24	1.12	1.21
F(000)	1384	1736	1328
μ/mm^{-1}	0.652	0.595	0.584
Reflections measured	3269	4535	3253
Unique reflections	3191	4434	3174
Reflections used	3182	4266	3157
R_1 /wR_2	0.039 / 0.056	0.047 / 0.059	0.032 / 0.051

4.3 NMR spectral measurements in $CDCl_3$ and D_2O solution

Twenty-five mg samples of each compound were dissolved in 0.5 mL of $CDCl_3$ or D_2O. All spectra were recorded on a JEOL Delta500 spectrometer. For all experiments, original JEOL pulse programs were used. Tetramethylsilane (TMS) was used as an internal standard. In the D_2O solution of **1**, pD was adjusted to 6.0 with the addition of 20% DCl.

^1H NMR spectral measurements were performed at 500.160 MHz and ^{13}C measurements at 125.765 MHz. The FG-DQF spectra were acquired in a 1 K × 0.5 K (F2 × F1) data matrix with 2 scans. The FG-HMQC spectra were acquired in a 0.5 K × 1 K [(F1(^{13}C) × F2(^1H)) data matrix with 4 scans. The FG-HMBC experimental data were acquired in a 0.5 K × 1 K (F1 × F2) data matrix with 8 scans for each experiment. The phase-sensitive NOESY spectra were acquired at 25 °C with a 1 K × 0.5 K data matrix and a mixing time of 550 ms. The ROESY spectrum for **1** in D_2O was acquired at 25 °C with a 1 K × 0.5 K data matrix and mixing time of 180 ms.

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