EPIMERIZATION OF ERYTHROMYCIN DERIVATIVES

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Dirithromycin (**3**) isomerizes upon dissolution in different solvents. From X-ray analysis of V-T 108, an analogue of dirithromycin, and comparative ^1H and ^{13}C NMR, and MS data, the isomer of dirithromycin was confirmed to be the C-16-(*S*)-epimer. The ratio of the two epimers at equilibrium conditions was approximately 8:2 (*R*/*S*) in methanol at room temperature.

To improve the therapeutic properties of erythromycin, a series of tetrahydro-1,3-oxazines **2** was synthesized by condensation of 9(*S*)-erythromycylamine **1** with substituted acetaldehydes[1].

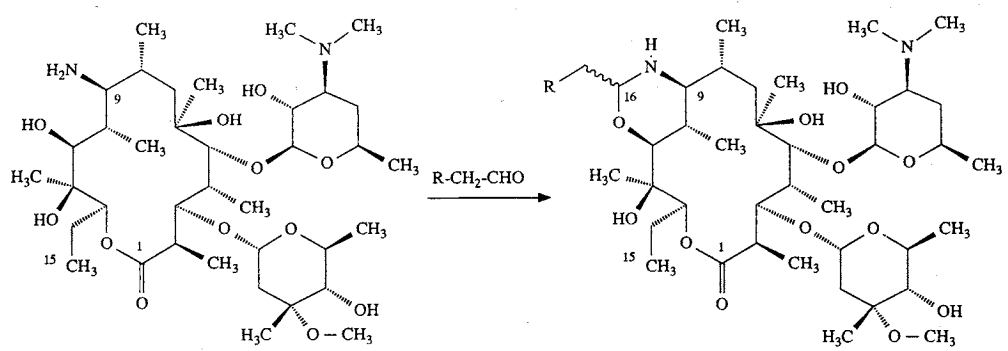
From these, dirithromycin (**3**) (**2**, R = $CH_3OCH_2CH_2O$–) was selected for further investigations. The structure of **3** was confirmed by X-ray analysis[2]. Thereby, the (*R*)-configuration of the newly introduced chiral center at C-16 could be established in dirithromycin.

Preliminary Observations

Crystallization of the condensation product of **1** with 2-methoxyethoxyacetaldehyde from acetonitrile gave pure **3**, as determined by X-ray analysis and ^1H NMR spectra.

Dissolution of **3** in different solvents leads to an equilibrium of **3** with a new compound **3a**, detected by TLC. The rate of formation of **3a** depends on solvent, time, and temperature. ^1H NMR investigations showed the equilibration rate in methanol to be low (approx 5% within 24 hours) compared to other solvents as acetone, chloroform or toluene.

The ratio of **3** to **3a** at equilibrium conditions at room temperature was estimated to be approximately 8:2. The isolation of pure **3a** by TLC failed because rechromatography of the isolated spot revealed again



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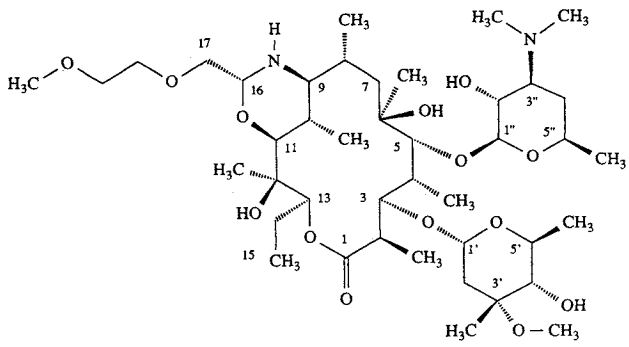
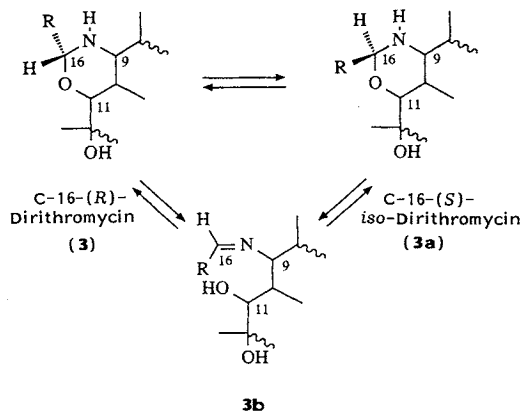


3

the formation of a mixture of **3** and **3a**.

For investigation by EI-MS the separated spots of **3** and **3a** were rapidly eluted from TLC plates with methanol at room temperature to avoid any further alteration of the compounds. The corresponding mass spectra taken from these samples were nearly identical. There were only some minor differences in the intensities of the occuring ions, thus suggesting that **3** and **3a** are stereoisomers.



3b

Stereoisomers can be formed most probably by epimerization of the newly generated chiral center C-16 of the tetrahydro-1,3-oxazine ring†.

This epimerization is comparable to the well-known formation of the anomeric equilibrium of α- and β-D-glucose by dissolving β-D-glucose.

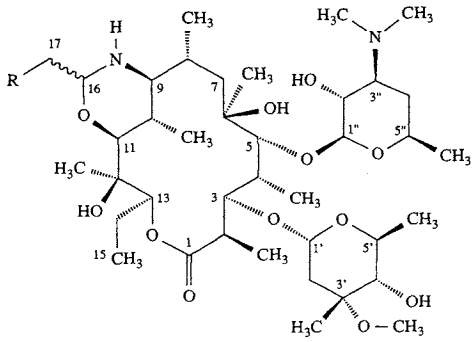
In all solvents tested so far *iso*-dirithromycin (**3a**) is the minor component. Since all attempts to isolate crystalline **3a** failed, the structure of **3a** could not be determined by X-ray analysis. Structure elucidation by NMR, however, is expected to be rather complex since an inversion of the configuration at C-16 probably may cause a change in the conformation of the whole molecule. Thus a structural proof for **3a** by NMR would at least require a conformational analysis of the macrolide ring. Albeit there are several methods for conformational analysis by NMR, their application will be rather difficult in this case, because **3a** can only be investigated as the minor component in a mixture with **3**. Therefore troublesome signal overlapping has to be expected in the NMR spectra.

An analogue of **3**, V-T 108 (**4**) (**2**, R=CH_3CONH-), also forms an equilibrium with a minor isomer **4a** in several solvents but with inverse chromatographic properties compared to **3**/**3a**. Therefore, if **4a** corresponds to **3**, and **4** to **3a**, respectively, **4** should have C-16(*S*)-configuration. In that case a similar conformation in solution and hence a close relation in the corresponding NMR spectra of **3a** and **4** could be expected, since both compounds differ only in their side chains at C-17.

The interpretation of the NMR spectra from the isomeric mixtures of **3**/**3a**, and of **4**/**4a**, is mainly

† The isomerization may also comprise the corresponding imino compound **3b**. However, its complete absence is proven by the ^{13}C NMR spectrum of the isomer mixture which lacks any resonance above 110 ppm except that belonging to the lactone carbonyl.

Table 1. ^1H Chemical shift assignments of dirithromycin (**3**), *iso*-dirithromycin (**3a**), V-T 108 (**4**), and *iso*-V-T 108 (**4a**) in $CDCl_3$.



Dirithromycin (**3**)	R = $\underset{20}{CH_3}-O-\underset{19}{CH_2}-\underset{18}{CH_2}-O-$
iso-Dirithromycin (**3a**)	R = $\underset{20}{CH_3}-O-\underset{19}{CH_2}-\underset{18}{CH_2}-O-$
V-T 108 (**4**)	R = $\underset{19}{CH_3}-\underset{18}{CO}-NH-$
iso-V-T 108 (**4a**)	R = $\underset{19}{CH_3}-\underset{18}{CO}-NH-$

Compound	δ (ppm) (n, am)[a] $J_{(H,H)}$ (Hz) Dirithromycin (**3**)	*iso*-Dirithromycin (**3a**)	V-T 108 (**4**)	*iso*-V-T 108 (**4a**)
2-H	2.72 (1H, m)	2.85	2.92	2.75
	$J_{2,3}=2.0$, $J_{2,2\text{-}CH_3}=7.3$	$J_{2,3}=9.5$, $J_{2,2\text{-}CH_3}=7$	$J_{2,3}=10.0$, $J_{2,2\text{-}CH_3}=7.3$	$J_{2,3}=2$, $J_{2,2\text{-}CH_3}=7.4$
2-CH_3	1.19 (3H, d)	1.19	1.20	1.19
	$J_{2\text{-}CH_3,2}=7.3$	$J_{2\text{-}CH_3,2}=7$	$J_{2\text{-}CH_3,2}=7.3$	
3-H	4.05 (1H, t[b])	4.24 (1H, dd)	4.34 (1H, d[b])	4.03 (1H, t[b])
	$J_{3,4}=2$	$J_{3,4}=1$	$J_{3,4}=<1$	$J_{3,4}=2$
4-H	1.75 (1H, m)	1.97[c]	1.96[c]	1.73
4-CH_3	1.10 (3H, d)	1.11[c]	1.08	1.05[c]
	$J_{4\text{-}CH_3,4}=7.4$		$J_{4\text{-}CH_3,4}=7.2$	
5-H	3.98 (1H, d)	3.6[c]	3.60	3.96
	$J_{5,4}=3.7$		$J_{5,4}=8.2$	$J_{5,4}=3.7$
6-CH_3[d]	1.13 (3H, s)	1.08[c]	1.10	1.13
7-Ha	1.4[c] (1H, m)	n.i.	1.5[c]	1.38[c]
7-Hb	1.3[c] (1H, m)	n.i.	1.2[c]	n.i.
8-H	2.1[c] (1H, m)	2.1[c]	2.0[c]	2.1[c]
8-CH_3	1.35 (3H, d)	1.00	0.96	1.32
	$J_{8\text{-}CH_3,8}=7$	$J_{8\text{-}CH_3,8}=7.2$	$J_{8\text{-}CH_3,8}=7.2$	$J_{8\text{-}CH_3,8}=7$
9-H	2.1[c] (1H, m)	2.58[c]	2.62 (1H, t[b])	2.19 (1H, d[b])
	$J_{9,8}=9$[e]	$J_{9,8}=J_{9,10}=<2$	$J_{9,8}=J_{9,10}=2$	$J_{9,8}=9$, $J_{9,10}=<2$
10-H	1.83 (1H, q[b])	1.9[c]	1.9[c]	1.84 (1H, q[b])
	$J_{10,10\text{-}CH_3}=6.7$			$J_{10,10\text{-}CH_3}=6.7$
10-CH_3	1.14 (3H, d)	1.09[c]	1.07	1.09
	$J_{10\text{-}CH_3,10}=6.7$		$J_{10\text{-}CH_3,10}=6.5$	
11-H	3.28 (1H, d)	3.7[c]	3.76	n.i.
	$J_{11,10}=1.4$		$J_{11,10}=2.1$	
12-CH_3[d]	1.08 (3H, s)	n.i.	1.24	1.10
13-H	4.92 (1H, dd)	5.05	5.14	4.92
	$J_{13,14a}=9.8$,	$J_{13,14a}=10.7$,	$J_{13,14a}=11.2$,	$J_{13,14a}=10.6$,
	$J_{13,14b}=2.6$	$J_{13,14b}=2.4$	$J_{13,14b}=2.0$	$J_{13,14b}=2.0$
14-Ha	1.45 (1H, m)	1.45[c]	1.49[c]	1.43[c]
14-Hb	1.94 (1H, m)	1.9[c]	1.95[c]	n.i.
15-H	0.89 (3H, t)	0.84	0.85	0.87
	$J_{15,14a}=7.5$,	$J_{15,14a}=7.5$,	$J_{15,14a}=7.5$,	$J_{15,14a}=7.5$,
	$J_{15,14b}=7.5$	$J_{15,14b}=7.5$	$J_{15,14b}=7.5$	$J_{15,14b}=7.5$
16-H	4.61 (1H, t[b])	4.66 (1H, dd)	4.67 (1H, t[b])	4.53 (1H, dd)
	$J_{16,17a}=3$,	$J_{16,17a}=3.8$,	$J_{16,17a}=2$,	$J_{16,17a}=3.5$,
	$J_{16,17b}=3$	$J_{16,17b}=6.9$	$J_{16,17b}=2$	$J_{16,17b}=7.5$
17-Ha	3.58[c] (1H, m)	3.6[c]	4.02 (1H, m)	3.2[c]
	$J_{17a,16}=3$	$J_{17a,16}=3.8$	$J_{17a,16}=2$, $J_{17a,17b}=14$	$J_{17a,16}=3.5$

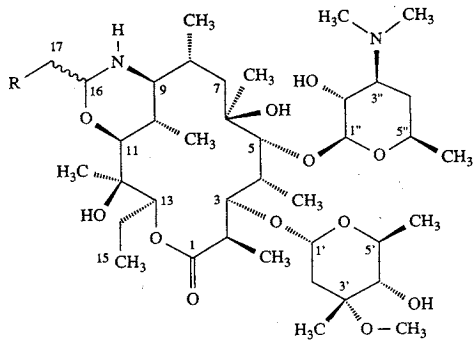
Table 1. (Continued)

Compound	Dirithromycin (3)	*iso*-Dirithromycin (3a)	V-T 108 (4)	*iso*-V-T 108 (4a)
	δ (ppm) (n, am)[a] $J_{(H,H)}$ (Hz)			
17-Hb	3.6[c] (1H, m) $J_{17b,16}=3$	3.47 (1H, m) $J_{17b,16}=6.9$, $J_{17b,17a}=9.7$	3.00 (1H, d[b]) $J_{17b,16}=2$, $J_{17b,17a}=14$	3.2[c] $J_{17b,16}=7.5$
17-NH	—	—	7.02 (1H, d) $J_{NH,17a}=9.2$	6.03
18-H	3.5~3.7 (2H, m)	3.5~3.7	—	—
19-H	3.5~3.7 (2H, m)	3.5~3.7	—	—
19-CH_3	—	—	2.12 (3H, s)	2.01
20-OCH_3	3.39 (3H, s)	3.38	—	—
1′-H	4.80 (1H, d) $J_{1',2'}=7.3$	4.44 $J_{1',2'}=7.3$	4.38 $J_{1',2'}=7.3$	4.80 $J_{1',2'}=7.3$
2′-H	3.32 (1H, dd) $J_{2',1'}=7.3$, $J_{2',3'}=10.4$	3.25 $J_{2',1'}=7.3$, $J_{2',3'}=10.2$	3.24 $J_{2',1'}=7.3$, $J_{2',3'}=10.2$	3.31 $J_{2',1'}=7.3$, $J_{2',3'}=10.0$
3′-H	2.54 (1H, m) $J_{3',2'}=10.4$, $J_{3',4'e}=4.0$, $J_{3',4'a}=12.0$	2.46[c]	2.45 (1H, m) $J_{3',2'}=10.2$, $J_{3',4'e}=3.8$, $J_{3',4'a}=12.0$	2.52 (1H, m) $J_{3',2'}=10.0$, $J_{3',4'e}=4.0$, $J_{3',4'a}=12.0$
3′-$N(CH_3)_2$	2.31 (6H, s)	2.30	2.29	2.31
4′-He	1.62 (1H, m) $J_{4'e,4'a}=13$, $J_{4'e,5'}=2$, $J_{4'e,3'}=4$	n.i.	1.62 $J_{4'e,4'a}=12.8$, $J_{4'e,5'}=2.2$, $J_{4'e,3'}=3.8$	1.64
4′-Ha	1.3[c] (1H, m)	n.i.	1.23[c]	n.i.
5′-H	3.6[c] (1H, m)	3.5[c]	3.43 (1H, m) $J_{5',4'e}=2.2$, $J_{5',4'a}=10.6$	3.64 (1H, m)
5′-CH_3	1.18 (3H, d) $J_{5'\text{-}CH_3,5'}=6.3$	1.20[c]	1.21 (3H, d) $J_{5'\text{-}CH_3,5'}=6.1$	n.i.
1″-H	5.22 (1H, d[b]) $J_{1'',2''a}=4.8$, $J_{1'',2''e}=1.2$	4.92 $J_{1'',2''a}=4.6$, $J_{1'',2''e}=<1.5$	4.83 $J_{1'',2''a}=4.7$, $J_{1'',2''e}=<1.5$	5.32 $J_{1'',2''a}=4.5$, $J_{1'',2''e}=<1.5$
2″-He	2.43 (1H, d[b]) $J_{2''e,2''a}=15.2$	2.37 $J_{2''e,2''a}=15$	2.35 $J_{2''e,2''a}=15.2$	2.44 $J_{2''e,2''a}=15$
2″-Ha	1.57 (1H, dd) $J_{2''a,1''}=5.1$	1.54 $J_{2''a,1''}=5$	1.55 $J_{2''a,1''}=4.7$	1.57 $J_{2''a,1''}=5.2$
3″-CH_3[d]	1.22 (3H, s)	n.i.	1.25	n.i.
3″-OCH_3	3.38 (3H, s)	3.32	3.31	3.38
4″-H	3.03 (1H, d) $J_{4'',5''}=9$	3.03 $J_{4'',5''}=9.5$	3.03 $J_{4'',5''}=9.5$	3.07 $J_{4'',5''}=9.5$
5″-H	3.90 (1H, m) $J_{5'',5''\text{-}CH_3}=6.2$	4.07 $J_{5'',5''\text{-}CH_3}=6$	4.05 $J_{5'',5''\text{-}CH_3}=6.3$	3.88 $J_{5'',5''\text{-}CH_3}=6.3$
5″-CH_3	1.25 (3H, d)	1.33	1.30	1.25

[a] n: Number of protons, am: appearant multiplicity of the proton signals. Only variations in the spectra are indicated in the respective columns.
[b] Signal of given multiplicity with unresolved fine structure.
[c] Overlapped resonance located by 2D-methods.
[d] The singlets of the methyl groups at position 6, 12 and 3″ could not be assigned with certainty.
[e] From spectrum in DMSO-d_6.
n.i.: Not identified.

based on the corresponding $^1H,^1H$-COSY and $^1H,^{13}C$-COSY spectra. The resulting assignments are shown in Tables 1 and 2. The assignment of some weaker signals is prevented by superpositions. They are indicated in the tables as "not identified". The ^1H and ^{13}C spectra of pure **3** and **4** were obtained from their freshly prepared solutions.

Table 2. ^{13}C Chemical shift assignments of dirithromycin (**3**), *iso*-dirithromycin (**3a**), V-T 108 (**4**), and *iso*-V-T 108 (**4a**) in $CDCl_3$.



Dirithromycin (**3**) R = $\underset{20}{CH_3}-O-\underset{19}{CH_2}-\underset{18}{CH_2}-O-$

iso-Dirithromycin (**3a**) R = $\underset{20}{CH_3}-O-\underset{19}{CH_2}-\underset{18}{CH_2}-O-$

V-T 108 (**4**) R = $\underset{19}{CH_3}-\underset{18}{CO}-NH-$

iso-V-T 108 (**4a**) R = $\underset{19}{CH^3}-\underset{18}{CO}-NH-$

Compound	δ (ppm)			
	Dirithromycin (**3**)	*iso*-Dirithromycin (**3a**)	V-T 108 (**4**)	*iso*-V-T 108 (**4a**)
1	176.98	175.29	176.63	177.49
2	44.48	44.89	45.23	44.76
2-CH_3	12.86	15.96	16.41	13.05
3	76.67	80.22	79.62	76.55
4	44.36	39.64	39.38	44.54
4-CH_3	8.96	9.28	9.16	8.84
5	78.99	84.11	83.95	78.98
6[a]	74.50	73.87	73.65	74.49
6-CH_3[b]	24.63	26.91	27.37	24.46
7	39.15	34.57	34.80	38.97
8	29.30	33.08	33.19	29.81
8-CH_3	20.75	21.45	21.34	21.03
9	65.67	64.04	63.94	66.12
10	27.50	28.69	28.72	27.77
10-CH_3	14.10	19.10	19.74	14.41
11	72.72	70.62	70.96	73.18
12[a]	74.39	74.06	73.95	73.94
12-CH_3[b]	14.83	15.46	15.37	14.68
13	76.33	76.10	76.38	76.14
14	21.32	21.21	21.00	21.03
15	11.19	10.59	10.51	11.05
16	82.63	82.63	82.94	83.41
17	72.81	73.84	42.94	43.66
18	70.82	70.62	173.12	n.i.
19	71.89	71.66	23.11	23.11
20	58.96	58.87	—	—
1′	100.88	103.20	103.42	100.93
2′	70.98	70.62	71.02	70.99
3′	64.88	65.40	65.49	64.96
3′-$N(CH_3)_2$	40.34	40.22	40.36	40.36
4′	28.80	29.44	28.69	28.69
5′	69.38	68.79	68.80	69.45
5′-CH_3	21.04	21.21	21.35	21.50
1″	94.27	96.39	96.38	94.26
2″	34.34	34.98	34.95	34.36
3″	72.67	72.70	72.63	72.63
3″-CH_3[b]	24.84	21.75	21.50	21.82
3″-OCH_3	49.16	49.41	49.43	49.16
4″	78.99	77.93	78.03	78.21
5″	65.91	65.40	65.30	66.03
5″-CH_3	18.32	18.49	18.50	18.21

[a] Interchangeable assignments.

[b] The chemical shifts of the methyl groups at position 6, 12 and 3″ could not be assigned unambiguously.

n.i.: Not identfied.

Results

Regarding the 1,3-oxazine structure the occurence of a signal for a strongly deshielded CH-group between 82.5 and 83.5 ppm (Table 2) in the ^{13}C NMR spectra of all four compounds can only be attributed to an $-O-CH(CH_2R)-NH-$ moiety. The same conclusion can also be derived from the ^{1}H NMR spectra where the signals of the proton at C-16 can be easily recognized by their coupling with the C-17 methylene group. These results clearly indicate that both pairs of compounds **3**/**3a** and **4**/**4a** are stereoisomers as suggested already by comparison of the corresponding EI mass spectra.

Dirithromycin (**3**) and its isomer **3a** show very different NMR spectra. Differences in chemical shifts are especially large for the protons at position 2, 3, 4, 5, 8-CH_3, 9, 11, 13, 1′ and 1″ and the carbon atoms 2-CH_3, 3, 4, 5, 7, 8, 10-CH_3, 11, 1′ and 1″. Since the vicinal coupling constants of the macrolide ring protons are also very different the conformation of the macrolide ring of the two isomers must be different. Therefore to prove the configuration at C-16 by NMR a complete conformational analysis is inevitable.

However, there is a striking consistency of the NMR data of **3a** with those of **4** (except for their different side chains at C-17) which renders the corresponding spectra practically identical. The chemical shift differences between corresponding protons are less than 0.1 ppm and the coupling constants are virtually identical within the accuracy of measurement (Table 1). Furthermore, the ^{13}C chemical shifts given in Table 2 which reflect structural differences even more sensitively are in accord within 0.5 ppm. The same consistency of NMR data also exists for **4a** and **3**.

These results unambiguously confirm the close conformational similarity of **3a** and **4** and as a consequence their identical chemical structures except the differences in the side chains at C-17. Thus X-ray analysis of crystalline **4** should provide final evidence for the structure of **3a** and consequently for the epimerization of **3** in solution.

Crystals of **4** suitable for X-ray analysis were obtained by crystallization from methanol. The X-ray analysis unambiguously confirmed the presence of a tetrahydro-1,3-oxazine moiety with (*S*)-configuration at C-16[3]. In addition, comparison with the X-ray analysis of **3** revealed the different overall conformation of both compounds especially in the 1,3-oxazine ring as concluded from the NMR spectra.

Conclusion

Dirithromycin (**3**) forms an equilibrium with its C-16-(*S*)-epimer **3a** in different solvents. Structural evidence for the epimerization was obtained by comparing **3**, V-T 108 (**4**), with their corresponding isomers **3a** and **4a**, on the basis of the ^{1}H and ^{13}C NMR spectra and the X-ray analysis of **3** and **4**. From these findings the following interrelation can be derived:

C-16-(*R*)-configuration:	Dirithromycin (**3**)	≡	*iso*-V-T 108 (**4a**)
	↓↑		↓↑
C-16-(*S*)-configuration:	*iso*-Dirithromycin (**3a**)	≡	V-T 108 (**4**)

Experimental

TLC was performed with precoated silica gel plates 60F 254 (Merck, Darmstadt, FRG) using *n*-pentane - dichloromethane - methanol - conc NH_3 (40 : 60 : 10 : 0.6) as a mobile phase. The spots were detected by UV light 254 nm or by spraying with molybdatophosphoric acid hydrate GR (Merck, Darmstadt, FRG) and subsequent heating of the plates to 120°C for 7 minutes. For mass spectrometric analysis the spots were eluted with methanol at room temperature.

EI mass spectra (70 eV) were performed with an CH5 mass spectrometer (MAT, Bremen, FRG) linked to an INCOS data system (Finnigan, San Jose, U.S.A.) using a direct insertion probe of standard design.

The evaporation temperature of the samples within the ion source was about 185°C.

^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100.58 MHz) were recorded on a Bruker WM 400 NMR spectrometer (Bruker, Karlsruhe, FRG) at room temperature using TMS as an internal standard ($\delta=0$). ^{1}H NMR spectra were taken from 0.02 mM solutions in $CDCl_3$, $CDCl_3$-CD_3OD (3:1), DMSO-d_6, DMSO-d_6-CD_3OD (3:1), CD_3OD, D_2O, and D_2O-buffer (pH=7.4). ^{13}C NMR spectra were recorded from 0.05 mM solutions in $CDCl_3$, DMSO-d_6, and CD_3OD. ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-COSY experiments were performed by application of standard Bruker DISNMR software using nearly saturated solutions in $CDCl_3$ and DMSO-d_6.

References

1) MAIER, R.; E. WOITUN, B. WETZEL & U. LECHNER: Synthesis, structure and biological activity of tetrahydro-1.3-oxazines derived from 9(*S*)-erythromycelamine and substituted acetaldehydes. Program and Abstracts of the 28th Intersci. Conf. on Antimicrob. Agents Chemother., No. 917, p. 273, Los Angeles, Oct. 23~26, 1988
2) LUGER, P. & R. MAIER: Molecular structure of 9-deoxy-11-deoxy-9-11-(imino(2-(2-methoxyethoxy)ethyliden)oxy)-(9S)-erythromycin, a new erythromycin derivative. J. Cryst. Mol. Structure 9: 329~338, 1979
3) LUGER, P.; A. PROX & E. WOITUN: Structure of a new erythromycin derivative, V-T 108. Acta Crystallogr, to submitted