CHEMICAL MODIFICATIONS OF THE ALIPHATIC BRIDGE OF ANSAMYCINS

SYNTHESIS AND ACTIVITY OF 25-DEACETOXY-25-*EPI*-HYDROXYRIFAMYCIN S

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Rifamycins are supposed to bind to, and inhibit the bacterial DNA-dependent RNA polymerase (DDRP) by the formation of hydrogen bonds through O (1), O (2), O (9), O (10). Therefore, with the aim of increasing the intrinsic activity of rifamycin S (**1**), the 25-deacetoxy-25-*epi*-hydroxyrifamycin S (**8**), was synthesized, which displays an additional hydroxyl available for the inhibiting interaction with the bacterial enzyme.

The configuration and conformation of the new compound were as expected, but the biological evaluation did not confirm the hypothesis.

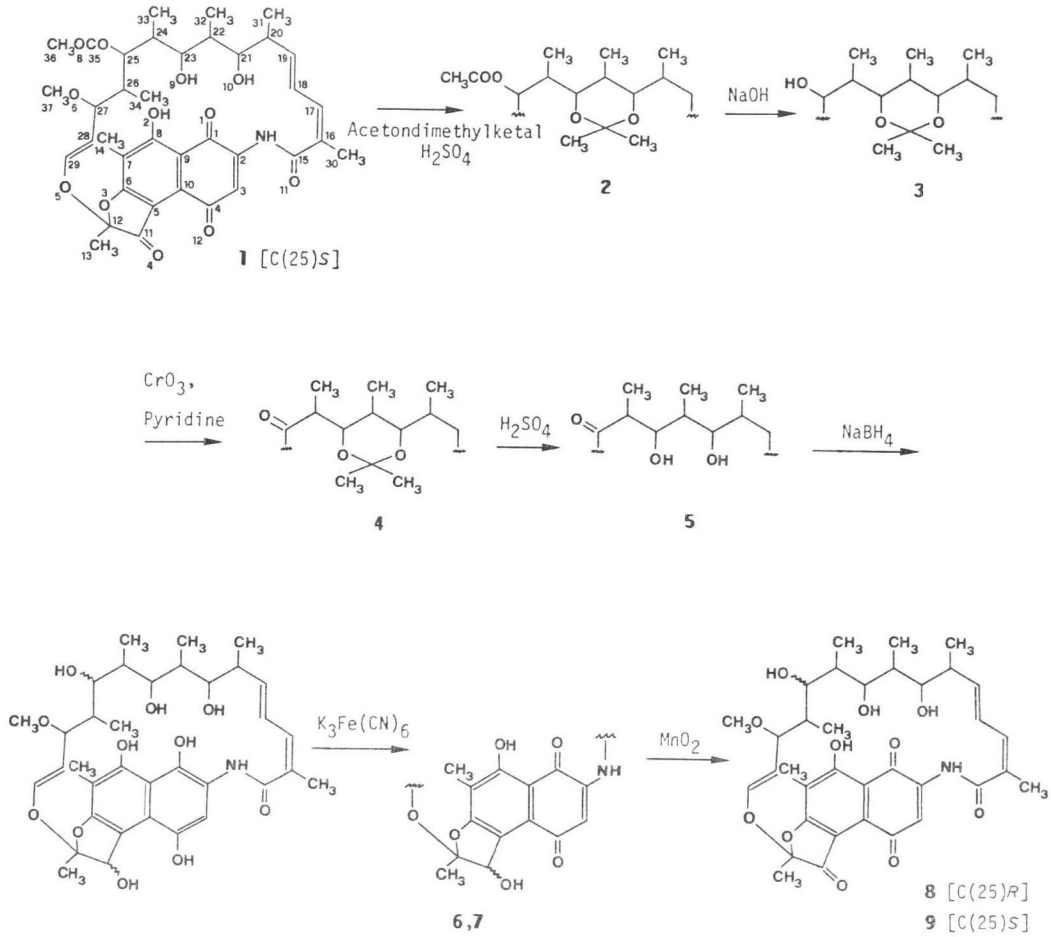
Extended chemical studies, together with the determination of the crystal structures of a few rifamycins allowed one to define the chemical and steric requisites necessary for the inhibitory activity of these antibiotics on the bacterial DNA-dependent RNA polymerase (DDRP)[1,2]. The results, recently confirmed by a ^{1}H NMR conformational study in solution[3], showed that the intrinsic activity is due to the presence of the underivatized oxygenated functions on C (1), C (8), C (21) and C (23) which display CO bonds parallel to the plane of the naphthoquinone-system, all pointing out on the same side of the molecule. These groups were then argued to be mainly responsible for the binding to the enzyme with which they share hydrogen bonds. According to this assumption we wanted to test the hypothesis that the creation of an additional center of possible hydrogen-bond interaction on the same side of the molecule could increase the intrinsic activity of rifamycins. In fact this could be realized by hydrolyzing the acetoxy group at C (25) and inverting the configuration of the same C (25).

Therefore the synthesis of 25-deacetoxy-25-*epi*-hydroxyrifamycin S (**8**), was carried out and is reported together with a ^{1}H NMR conformational study in solution. The activities both on cell-free isolated enzyme and *in vitro*, are also reported, in comparison with that of the epimeric 25-deacetoxy-25-hydroxyrifamycin S (**9**)[4].

Chemistry

Since we were unable to perform the direct inversion of configuration at C (25) in rifamycins, we planned to carry out the preparation of **8**, starting from rifamycin S (**1**), as outlined in Scheme 1. The intermediate **5** was already known and its preparation was carried out as described in the reference[4]. The treatment of **5** with sodium borohydride brought then the reduction of the quinone nucleus to hydroquinone, and of the carbonyls at C (11)[5] and C (25). The rifamycinol-like derivatives thus obtained, in which the carbonyl group at C (11) is reduced to secondary alcohol, are unstable in the hydroquinone form, and were reoxidized to quinone by treatment with potassium ferricyanide[5]. A further

Scheme 1.

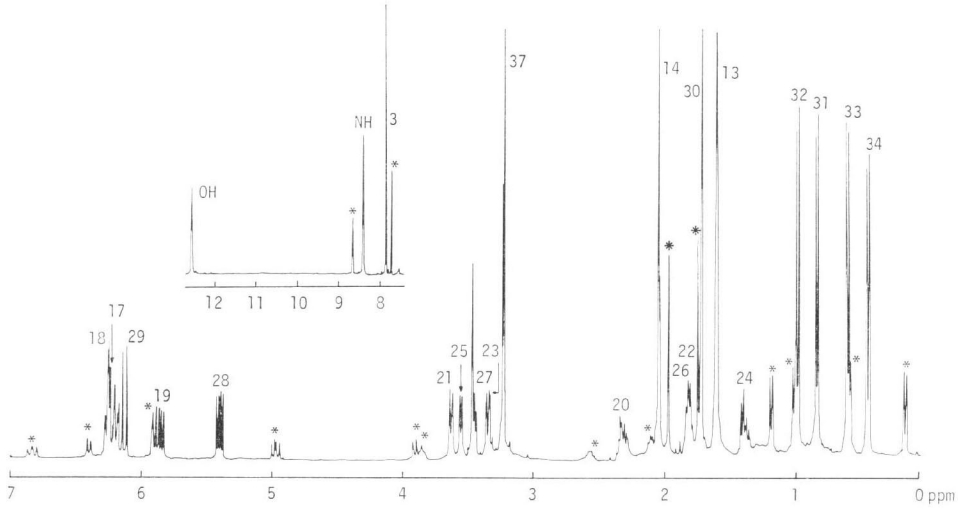


treatment with manganese dioxide reformed the carbonyl at C (11) giving a mixture of the epimers **8** and **9**. Finally, **8** was isolated and identified; **9** was also isolated and shown to be identical with a sample obtained directly by alkaline hydrolysis of **1**[4].

Synthesis

500 mg of **5** was dissolved in 40 ml of absolute ethanol, and 750 mg of sodium borohydride was added gradually. After 4 hours 40 ml of methanol was added. Under vigorous stirring a 33% solution of potassium ferricyanide in water was added, and the mixture was then acidified with citric acid and extracted with chloroform. The chloroform extract, dried and evaporated, gave a residue of **6** and **7**, 350 mg, which was dissolved in 10 ml of acetonitrile and treated for 72 hours with manganese dioxide. After filtration the solution was evaporated and the residue (**8**, **9**) was purified on preparative silica gel plates (Merck 60 F_{254} 20×20×0.25 cm) developed with chloroform - ethyl acetate, 8: 2. Two fractions were recovered: one **8**, 200 mg, corresponding to Rf 0.70, crystals from ethyl acetate - petroleum ether, mp 148~149°C; the other **9**, 80 mg, corresponding to Rf 0.20. NMR and elemental analyses of the isolated compounds were consistent with the formulae.

Fig. 1. 400 MHz spectrum of **8** in $CDCl_3$ (ppm from Me_4Si).
* Minor isomer.



NMR

The 400 MHz spectrum of **8** in $CDCl_3$ is shown in Fig. 1. Two sets of lines were clearly observable, but after a trace of acetic acid was added one set remained, coincident with the major set.*

Chemical shifts, coupling constants and assignments for the major set are reported in Table 1 for **1**, **8**, and **9**. The intensity ratios between the two observed rotational isomers in $CDCl_3$ were 2: 1 in **1**, 2: 1 in **8**, and 9: 1 in **9**.

Four protons exchangeable with D_2O were present in **1**, while they were five in **8** and **9**. By examining the chemical shifts reported in Table 1, one can observe that there are strong variations relative to H (23), H (24), H (25) and H (26) in both **8** and **9** with respect to **1**. In detail there is a $\Delta\delta$ of 0.62, 0.71, 1.45, 0.45 ppm respectively for **9**, and of 0.35, 0.60, 1.07, 0.30 ppm respectively for **8**, all other signals varying within a range of 0.20 ppm. The corresponding vicinal coupling constants are rather similar in the three compounds with the exception of H (24)–H (25) and H (25)–H (26), these are respectively 2.5 and 11 Hz in **1**, 2 and 10.5 Hz in **9**, and 6.7 and 1.7 Hz in **8**.

The correlation of the vicinal coupling constants with an optimized Karplus type equation[8] allowed one to derive the following dihedral angles: H (24)–H (25) $-60°$ in **1** and **9**, 150° in **8**; H (25)–H (26) 170° in **1** and **9**, $-75°$ in **8**.

The amide carbonyl is transoid with respect to C (2)–C (3) in **1**, **8** and **9**, since H (18) chemical shifts are in the range 6.2~6.3 ppm, while the carbonyl in the cisoid conformation would induce a deshielding effect on H (18) and the chemical shift would fall in the range 6.7~7.2 ppm. At the other end of the ansa chain the dihedral angle H (27)–C (27)–C (28)–H (28) is about 140° in **1** and **8** allowing a measurable long-range coupling constant H (27)–H (29) of 1.5 Hz, and about $-15°$ in **9**[8].

The 50.28 MHz ^{13}C NMR spectra of **1**, **8**, and **9** in $CDCl_3$ have been interpreted according to the literature; chemical shifts and tentative assignments are listed in Table 2. The spectra appear consist-

* As previously described[8] rifamycins display a rather rigid conformation along the ansa chain, but can give rise to four kinds of isomers deriving from the rotation of the amide plane and of the plane of the double bond C (28)=C (29). That is, the amide carbonyl can be either transoid or cisoid with respect to C (2)–C (3), and the dihedral angle H (27)–C (27)–C (28)–H (28) can be either about 150° or about 30°. When a trace of acid is added only one isomer appears.

Table 1. ^{1}H NMR data of **1**, **8**, and **9** in $CDCl_3$ (ppm from Me_4Si).

8 *J*(Hz)		**9** *J*(Hz)		**1** *J*(Hz)			**1**	**9**	**8**
8.40		8.46		8.5		H–N			
						C_{15}=O			
						C_{16}–$^{30}CH_3$	2.02	2.02	2.02
6.19		6.29		6.25		HC_{17}			
	12				10				
6.24		6.29		6.31		HC_{18}			
	14.8		14.5		16				
5.85		5.79		5.93		HC_{19}			
	7.33		6.4		7.5				
2.31		2.36		2.27		HC_{20}–$^{31}CH_3$	0.82	0.82	0.83
	10		10		10				
3.62		3.56		3.55		HC_{21}–OH			
	2		2		0				
1.80		1.78		1.78		HC_{22}–$^{32}CH_3$	1.00	1.03	0.98
	2.33		2		2				
3.33		3.60		2.98		HC_{23}–OH			
	10		10		12				
1.36		1.25		1.96		HC_{24}–$^{33}CH_3$	0.65	0.58	0.60
	6.7		2		2.5				
3.53		3.15		4.60		HC_{25}–OX	2.02		
	1.7		10.5		11				
1.80		1.95		1.50		HC_{26}–$^{34}CH_3$	0.20	0.18	0.45
	4.66		3.9		3				
3.43		3.45		3.37		HC_{27}–$^{37}OCH_3$	3.10	3.19	3.21
	6.67		10		8				
5.41		5.12		5.06		HC_{28}			
	13.4		13		12.5				
6.12		6.40		6.20		HC_{29}			
						O			
7.80		7.80		7.80		^{3}CH			
2.24		2.27		2.33		$^{13}CH_3$	X=$^{35}CO^{36}CH_3$	**(1)**	
1.71		1.69		1.72		$^{14}CH_3$	X=H	**(8)**	
12.60		12.50		12.50		OH	X=H	**(9)**	
	1.5		0		1.5	$J_{H_{27}-H_{29}}$			

ent with the formulae; in particular, the peaks assigned to C (35) and C (36) in **1**, are missing in the spectra of **8** and **9**.

These findings, together with the consideration that several rifamycins, studied by ^{1}H NMR in solution[3], displayed very little variation both in chemical shifts and vicinal coupling constants for the region C (20)–C (25), prove that **8** is in fact 25-deacetoxy-25-*epi*-hydroxyrifamycin S. Its conformation along the ansa chain is similar to that of rifamycin S (**1**), but the configuration of C (25) is opposite.

Activity on Isolated DDRP

The inhibition tests of **1**, **8** and **9** on isolated DDRP from *E. coli* B (EC 2.7.7.6) were performed according to standard procedures[10]. A concentration of enzyme 10 nM, and concentrations of antibiotic up to 300 nM were used. At the highest concentration **1** gave about 90% inhibition, while **8** and **9** gave about 50% inhibition.

Conclusions

The synthesis of **8** was planned with the aim of providing an additional hydroxyl on the same side

Table 2. ^{13}C NMR data for **1**, **9** and **8** in $CDCl_3$ (ppm from Me_4Si).
Assignment according to references 7, 8 and 9 for **1** and **9**, tentative assignment for **8**.

	1	9	8		1	9	8
C (1)	184.6	184.6	185.0	C (20)	38.8	39.7	40.4
C (2)	139.0	138.8	139.1	C (21)	73.3	71.1	73.4
C (3)	117.1	116.6	117.2	C (22)	32.6	32.4	32.9
C (4)	182.0	182.0	182.8	C (23)	77.1	77.1	83.0
C (5)	110.8	111.0	111.2	C (24)	37.3	37.6	39.9
C (6)	172.4	171.1	173.0	C (25)	73.3	72.3	72.0
C (7)	115.7	115.4	116.9	C (26)	37.3	37.6	36.7
C (8)	166.7	166.8	167.0	C (27)	81.5	85.1	81.6
C (9)	110.6	110.5	110.5	C (28)	115.7	112.8	115.3
C (10)	130.5	130.6	132.3	C (29)	144.8	147.7	142.3
C (11)	191.7	191.2	192.3	C (30)	20.1	20.1	20.0
C (12)	108.3	109.0	107.9	C (31)	16.8	16.5	16.7
C (13)	22.1	22.9	21.9	C (32)	11.6	12.4	11.2
C (14)	7.5	7.5	7.5	C (33)	8.8	8.2	9.4
C (15)	169.5	169.3	169.8	C (34)	11.6	11.6	10.5
C (16)	130.7	131.2	132.4	C (35)	173.0	—	—
C (17)	133.8	133.2	132.5	C (36)	21.0	—	—
C (18)	124.0	123.6	123.7	C (37)	56.7	56.1	56.8
C (19)	142.1	141.1	142.2				

of the molecule as O(1), O(2), O(9), and O(10). This should have increased the stability of the enzyme-antibiotic complex by allowing a new hydrogen-bond to form. The synthesis of **8** was thus performed and the identity of the compound proved by NMR in solution which also allowed a conformational analysis of the molecule in comparison with **1** and **9**. The conformation of **8** appears as shown in Fig. 2. In the region C (20)–C (25) the conformation of the molecule appears to be the same as found in

Fig. 2. Dreiding model of **8** based on NMR data.

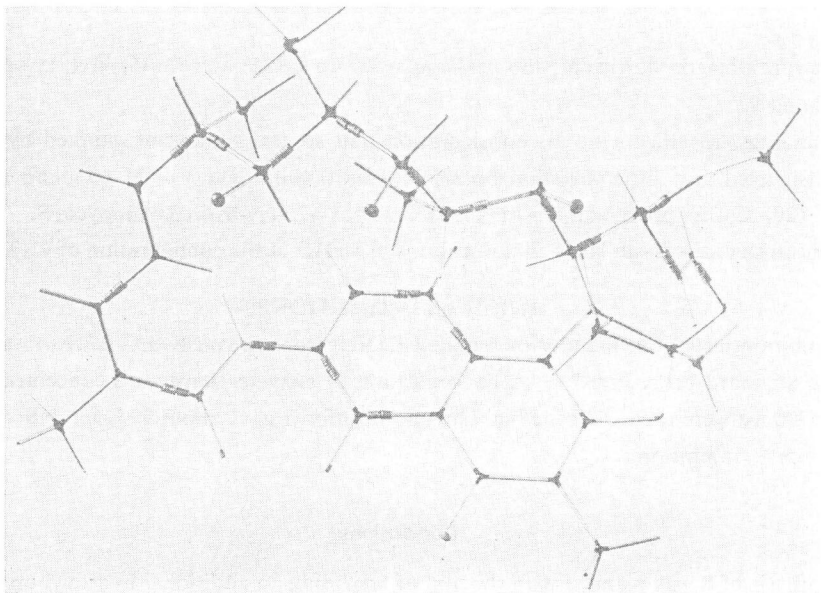


Table 3. Sensitivity of microorganisms (10^4 cells/ml) to **1**, **8** and **9**.

Microorganism	MIC (μg/ml)		
	1	**8**	**9**
Pseudomonas aeruginosa clinical isolate	>50	>50	>50
Escherichia coli clinical isolate	>50	〃	〃
Klebsiella pneumoniae clinical isolate	25~50	〃	〃
Proteus vulgaris clinical isolate	25~50	〃	〃
Salmonella typhi LP	>50	〃	〃
Shigella sonnei ATCC 9290	50	〃	〃
Staphylococcus aureus FDA 209P	0.005	0.05	0.05

Culture medium BHI.

many rifamycin S derivatives, and it is also the conformation expected to allow a stronger interaction with the bacterial enzyme due to the transformation effected at C (25). Nonetheless the cell-free tests on isolated DDRP showed that the intrinsic activity of **8** is similar to that of the epimeric **9** and lead to the conclusion that the inversion of configuration at C (25) in rifamycins does not contribute to the enzyme-antibiotic binding.

Furthermore, comparison of the Rf values of **8** and **9** showed that **9**, with C (25) in the natural *S* configuration, is more polar than **8**, the corresponding C (25) *R* isomer, the difference being probably due to a different ability to form intramolecular hydrogen bonds. Since **8**, and **9** displayed also a ΔRm[11] of 1.46, their antibacterial activities *in vitro* were measured in order to discover if the difference in the chromatographic behaviour, indicating a difference in the overall polarity of the molecules, could result in a difference in the ability to penetrate the bacterial cell-wall. The results, shown in Table 3, indicate that, like for the cell-free tests, the activities *in vitro* of **8** and **9** are the same.

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