CHEMICAL MODIFICATIONS OF THE ALIPHATIC BRIDGE OF ANSAMYCINS

3. SYNTHESIS AND ACTIVITY OF 21-*EPI*-RIFAMYCIN S

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Rifamycins inhibit bacterial DNA-dependent RNA polymerase through the formation of non-covalent bonds by the oxygenated groups at C(1), C(8), C(21), and C(23). These must be unhindered and underivatized, with the antibiotic in a proper overall molecular conformation. The present study shows that contrary to previous conclusions the availability of the hydroxyl group at C(21) is not as important as that of the other three groups.

In support of this is the observation that 21-*epi*-rifamycin S is partially active, both on the isolated DNA-dependent RNA polymerase and on some Gram-positive bacterial strains.

Rifamycin S (**1**, Scheme 1) belongs to a family of antibacterial antibiotics which specifically inhibit the bacterial enzyme, DNA-dependent RNA polymerase (DDRP), mainly by the formation of non-covalent bonds through O(1), O(2), O(9), and O(10)[1,2]. In order to have maximal activity, these four oxygenated functions must be unhindered and underivatized, and must display well-defined spatial relationships with one another[1,2]. Earlier studies[3] showed that the hydrolysis of the acetoxy group at C(25) in **1** as well as inversion of configuration at C(25), bringing an additional hydroxyl group on the same side as O(1), O(2), O(9), O(10), have no influence on activity. A further study showed that, on the contrary, an inversion of configuration at C(23) in **1** bringing, O(9) to the opposite side of the molecule with respect to O(1), O(2) and O(10), leads to an inactive compound. The present study reports the synthesis and the testing of the activity of 21-*epi*-rifamycin S (**3**) in which O(10) is made unavailable for the interaction of **1** with the bacterial enzyme.

Chemistry

Compound **3** was prepared as shown in Scheme 1. The oxidation of **1** with a 4: 1 excess of pyridine dichlorochromate (PDC) in DMF gave a mixture of C(21) (**2**) and C(23) (**2a**) monoketo derivatives, rather than the diketo derivative described in the literature[5]. The two monoketo derivatives were isolated by column chromatography on Silica gel 60 Merck, 40~63 μm, eluting with benzene - ethyl acetate, 7: 3. Yields: **2** 20% and **2a** 16%. Then **2** was reduced with $NaBH_4$ at C(1)~C(4), C(11), and C(21). Since the rifamycinols are less stable than the corresponding rifamycins, the products were immediately reoxidized at C(1)~C(4) with aqueous 33% potassium ferricyanide, and at C(11) with MnO_2. A mixture of **1** (yield 7.5%) and **3**, the C(21) epimer of **1**, (yield 50%) was obtained,

Table 1. ^{1}H NMR data of **2**, **2a**, **3** and **3a** in $CDCl_3$ at 200 MHz, δ (ppm from Me_4Si), J (Hz).

2	2a	3	3a		3a	3	2a	2
8.30	8.29	8.39		H–N$_{15}$				
				C–O				
				C$_{16}$–CH$_3$ (30)	2.06	2.04	2.05	2.05
				C$_{17}$				
6.28	6.27	6.32	6.20	HC				
(11.0)	(8.8)	(10.5)	(~10)	$_{18}$				
6.15	6.05	6.23	6.15	HC				
(13.5)	(15.5)	(11.4)	(~16)	$_{19}$				
5.60	5.69	5.71	5.81	HC				
(9.0)	(8.2)	(8.1)	(5.6)	$_{20}$				
3.22	2.10	2.24	2.29	HC$_{21}$–CH$_3$ (31)	0.88	1.03	0.92	1.14
—	(7.7)	(7.3)	(10.2)					
—	3.43	3.32	3.34	HC$_{22}$–OH	—	~3.8	—	—
—	(3.1)	—	(~3)					
2.78	2.60	1.75	1.57	HC$_{23}$–CH$_3$ (32)	0.87	1.00	0.98	1.08
(2.0)	—	(~2)	(~8)					
2.96	—	2.89	3.45	HC$_{24}$–OH	—	~3.8	—	3.37
(11.0)	—	(10.3)	(~1)					
1.60	2.71	1.75	1.85	HC$_{25}$–CH$_3$ (33)	0.78	0.53	1.04	0.58
(1.5)	(4.4)	(~2)	(2.9)					
4.92	4.89	4.72	4.70	HC$_{26}$–OAc (36)	2.01	2.03	1.95	2.04
(10.5)	(8.1)	(9.7)	(10.3)					
1.20	1.57	1.65	1.41	HC$_{27}$–CH$_3$ (34)	0.32	0.26	0.50	0.17
(3.0)	(4.0)	(2.2)	(~3)					
3.34	3.18	3.27	3.43	HC$_{28}$–OCH$_3$ (37)	3.06	3.07	3.03	3.06
(7.0)	(7.8)	(8.8)	(6.7)					
5.23	5.17	5.16	5.15	HC				
(12.5)	(12.6)	(10.1)	(12.5)	$_{29}$				
6.17	5.99	6.19	6.02	HC				
				O				
				^{3}C–H	7.68	7.88	7.66	7.87
				^{13}C–H	1.74	1.73	1.74	1.76
				^{14}C–H	2.26	2.31	2.27	2.30
				^{2}OH	12.47	12.59	12.49	12.52

In parenthesis are indicated J values.

which were isolated by chromatography as above.

The C(23) epimer of **1**, 23-*epi*-rifamycin S (**3a**) was also prepared, with similar yields, by a similar reaction-sequence.

FAB-MS gave M^+: 694 for **2** and **2a**, and M^+: 696 for **3** and **3a**.

The ^{1}H NMR spectra of **2**, **2a**, **3**, **3a** were registered at 200 MHz on a Brucker WP 200 instrument.

All signals corresponding to the proposed formulas were found and assigned on the basis of previous studies[3,4,6] (Table 1). In particular, the correlation of the vicinal coupling constants with an optimized Karplus type equation[7] allowed us to derive the following dihedral angles in **3**: H(20)-H(21) ~20°, H(21)-H(22) ~130°.

Activity

The inhibitory activities of **3** and **3a** on DDRP from *Escherichia coli* MRE 600 (Boehringer,

Scheme 1.

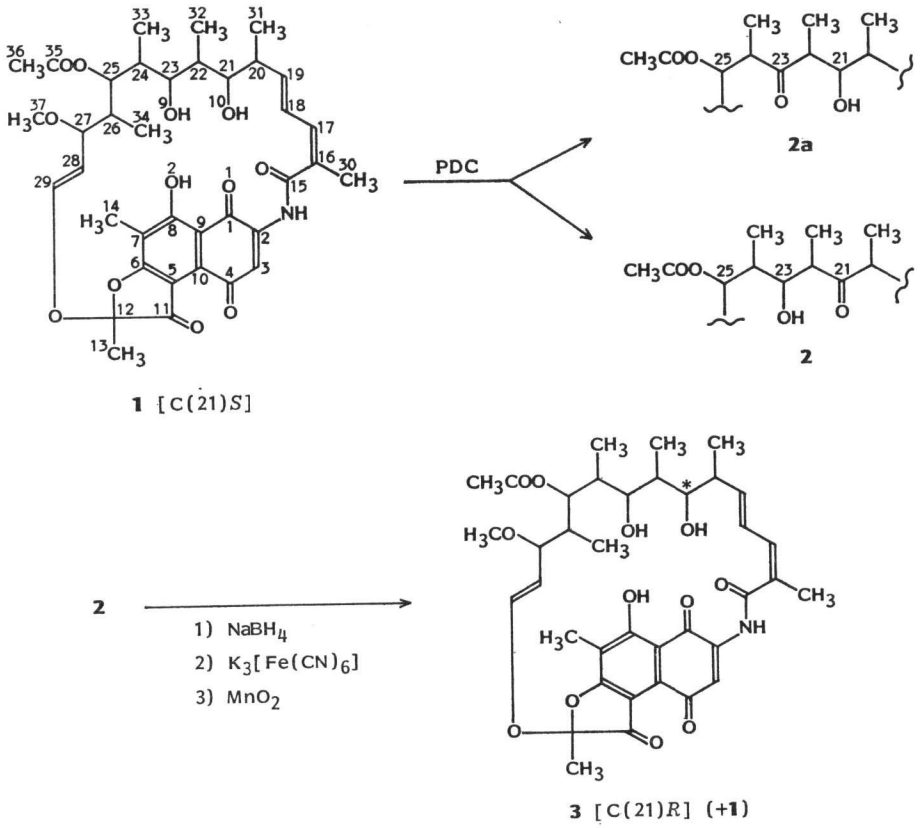

Table 2. Sensitivity of microorganisms (10^5 cells/ml) to **1** and **3**.

Microorganism	MIC (μg/ml)	
	1	**3**
Escherichia coli ML/35	6~12	50
E. coli enteropathogen O 124 N 221 LB	6~12	50
Klebsiella pneumoniae Barboni	12~25	50
Klebsiella Malpighi	6~12	50
Salmonella Livingstone	6~12	50
Salmonella Wien LP	12~25	50
Pseudomonas Malpighi	12~25	50
Proteus mirabilis XCR	6	50
Staphylococcus aureus Colliva	0.005	1.9~3.8
S. aureus 209 P (FDA)	0.005	5

Ingelheim) were compared to that of **1** using standard procedures[8]. One unit of enzyme and antibiotics in the range 10~50 nM were used per assay. At the highest concentration the enzyme was inhibited 70% by **1**, 25% by **3**, and was not inhibited by **3a**.

The antibacterial activity of **1** and **3** was also tested *in vitro* (Table 2) and **3** resulted slightly active on a few Gram-positive bacterial strains.

Conclusions

In conclusion, the inversion of configuration at C(21) in rifamycin S causes a net decrease, but not a complete loss of activity as is the case with the removal of the hydroxyl at C(1)[9] or at C(8)[10], and the inversion of configuration at C(23). With both 21-*epi*- and 23-*epi*-rifamycin S the epimer has lost one of the points of interaction of the ansa-chain with the bacterial enzyme, but in the latter case the structural variation leads to more drastic inactivation. This fact indicates a differential contribution by the two hydroxyls to the inhibitory binding.

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