RIFAMYCIN R, A NOVEL METABOLITE FROM A MUTANT OF *NOCARDIA MEDITERRANEA*

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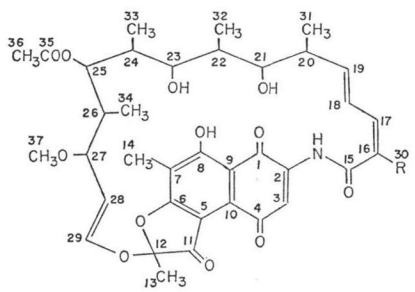
Rifamycin R is a novel ansamycin produced by a mutant of *Nocardia mediterranea*; both physical and chemical data indicate that it is 30-demethyl-30-hydroxymethyl rifamycin S.

Previous studies in our laboratories have indicated the importance of mutation of the rifamycin producer, *Nocardia mediterranea*, as a means of obtaining novel biosynthetic variants of the rifamycin molecule[1]. In a preliminary communication[2] we described the isolation of three new ansamycins (rifamycins P, Q and R) from the fermentation broth of a mutant of *N. mediterranea*. This mutant, along with two others that produce a similar mixture of rifamycins[3], has been deposited in the American Type Culture Collection (ATCC 31066; ATCC 31064 and ATCC 31065). We now report the structure elucidation of one of these ansamycins, rifamycin R (**I**). The structure of the others will be published elsewhere.

Structure Determination

Evidence for the structure of rifamycin R was obtained by comparing its physico-chemical data with those of the known compound rifamycin S (**II**). Elemental analysis of **I** accounts for the molecular formula $C_{37}H_{45}NO_{13}$. This is indicated by the molecular ion at *m/e* 711, obtained by field desorption mass spectrometry**, *i.e.* 16 a.m.u. more than that of **II** (*m/e* 695). Thus, rifamycin R contains one oxygen atom more than rifamycin S.

The UV-visible spectrum of **I** in MeOH [λ_{max}, nm (ϵ): 281 (29,400); 340 (8,100); 410 (5,100)] is the same as that of **II**[5], indicating that the extra oxygen has not modified the naphthoquinone chromophore. The IR spectra of **I** in nujol mull and $CDCl_3$ solution are rather similar to those of **II**[6] and show that the main structural features (amide moiety, chromophore and ansa chain from C-21 to C-25) of **I** and **II** are the same. The ^{1}H-NMR data of **I** in $CDCl_3$, obtained at 100 MHz by ^{1}H homodecoupling, are reported in the table. Comparison of these data with those reported for **II**[7] indicates



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** These data were obtained by courtesy of Varian MAT GmbH, Bremen, according to the procedure described in ref. 4. Unlike rifamycin S, rifamycin R did not give the molecular ion by electron impact.

that (a) the signal at δ 2.01 integrates for three protons, instead of six in **II** (protons assigned to CH_3-30 and CH_3-36); (b) there are two new 1H doublets at δ 4.20 and 4.40 ($J_{gem}=13$ Hz); (c) there is a new mobile proton at δ 7.86; (d) the conformation of the ansa chain of **I** is substantially the same as **II**, as shown by the chemical shifts and the vicinal interproton coupling constants of the ansa protons.

From all the previous data it is possible to postulate for rifamycin R the structure of hydroxy rifamycin S either at CH_3-30 (methyl on carbon 16 of the ansa) or at CH_3-36 (methyl of the acetoxy group at carbon 25).

The presence of the structural unit C (17) H=C (16)-CH_2(30) OH is indicated in the ^{1}H-NMR spectrum by: (a) D_2O exchange which reveals a small coupling ($J<1$ Hz) between OH-30 and CH_2-30; (b) ^{1}H homodecoupling experiments which indicate an allylic coupling between H-17 and the two nonequivalent protons of CH_2-30; this long range interaction is also present in **II** between H-17 and CH_3-30. Thus rifamycin R is 30-hydroxy-rifamycin S.

Biosynthetic studies with rifamycin S have shown that C-30 derives from the methyl group of propionic acid[8]. It seems that rifamycin R is biosynthesized from rifamycin S by oxidation of the methyl at C-30; an analogous modification of a propionate-derived methyl has already been established for rifamycin W[9,10]. The antibacterial activity of rifamycin R is similar to that of rifamycin S[3].

Experimental

UV-Vis spectra were measured on a Perkin Elmer 4000 and IR spectra on a Perkin Elmer model 421 spectrometer. ^{1}H-NMR spectra and ^{1}H homodecouplings were run on a Varian XL-100 instrument equipped with FT accessory.

Occurrence and isolation of rifamycin R

The mutant producing rifamycin R (ATCC 31066) was obtained by treating spores of *N. mediterranea* (ATCC 13685) with nitrosoguanidine and selecting for surviving colonies that produced antibacterial activity as previously described[3,11]. The new rifamycins were produced by fermentation of the above mutant in a complex organic medium[12] for 200 hours at 28°C. Fermentation broths were

Table 1. ^{1}H-NMR data of rifamycin R in $CDCl_3$ at 100MHz.

Proton	Multiplicity	δ(ppm)	J(Hz)	Proton	Multiplicity	δ(ppm)	J(Hz)
NH	s	9.41	—	H-25	dd	4.84	$J_{25,26}=10.0$
H-3	s	7.80	—	H-26	m	1.9	$J_{26,27}=2.0$
OH-8	s	12.50	—	H-27	dd	3.44	$J_{27,28}=6.0$
H-13	s	1.72	—	H-28	dd	5.07	$J_{28,29}=12.5$
H-14	s	2.32	—	H-29	d	6.10	
H-17	m	6.2~6.7	n.d.	H-30	2d	$\nu_A=4.20$	$J_{gem}=13$
H-18	m	6.2~6.7	n.d.			$\nu_B=4.40$	
H-19	m	6.2~6.7	n.d.	OH-30	s	7.86	
H-20	m	2.30	$J_{20,21}=9.5$	H-31	d	0.86	7
H-21	dd	3.62	$J_{21,22}=1.0$	H-32	d	1.00	7
OH-21	b	3.9		H-33	d	0.69	7
H-22	m	1.7	$J_{22,23}=1.5$	H-34	d	0.20	7
H-23	dd	3.00	$J_{23,24}=10.0$	H-36	s	2.01	—
OH-23	b	3.9		H-37	s	3.10	—
H-24	m	1.5	$J_{24,25}=1.0$				

b=broad; n.d.=not determined.

filtered, adjusted to pH 2.0 and extracted with ethyl acetate. Rifamycin R was separated from the other rifamycins by their extraction into 10 mM sodium phosphate buffer pH 7.38. The purification was carried out by column chromatography on silica gel Merck 0.05~0.2 mm, eluting with $CHCl_3$ - MeOH (99 : 1) and then by preparative TLC, using silica gel plates Merck 60 F_{254} and eluting with $CHCl_3$ - MeOH (95 : 5). Dark yellow crystals of pure rifamycin R were obtained by crystallization from ethyl acetate.

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