RIFAMYCIN Z, A NOVEL ANSAMYCIN FROM A MUTANT OF *NOCARDIA MEDITERRANEA*

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Rifamycin Z is a novel ansamycin produced by a mutant of *Nocardia mediterranea*. Physico-chemical data indicate that it possesses a lactone-type structure directly derived from rifamycin W.

During our studies on mutant strains of the rifamycin producer *Nocardia mediterranea*, we isolated a morphological variant which produced a mixture of novel ansamycins. We report the structure elucidation of the major component, rifamycin Z, together with its proposed biogenetic relationship with the ansamycins so far reported.

Structure Determination

Evidence for the structure of rifamycin Z (**I**) was obtained by comparing analytical and spectroscopic properties with those previously reported for rifamycin W[1] (**II**). Elemental analyses and a molecular ion in a EI mass spectrum at $m/z=651$, as well as ^1H and ^{13}C NMR spectral data indicate **I** has a molecular formula $C_{35}H_{41}NO_{11}$, *i.e.* **I** contains four hydrogens less than **II** ($C_{35}H_{45}NO_{11}$). The UV-VIS spectrum of **I** in pH 7.38 buffer solution [λ_{max}, nm (ε): 292 (21,700), 348 (10,800), 430 (sh), 550 (2,300)] is the same as that of **II**, indicating that the naphthoquinone chromophores in **I** and **II** are identical. This is confirmed by the mass spectrum of **I**, which shows a similar pattern of fragmentation of the chromophore as that of **II**, *i.e.* fragments at $m/z=286$, 274, 273, 258 and 246. The mass fragmentation attributable of the ansa chain indicated a loss of two molecules of water instead of four as in **II**[2], thus suggesting that the structural difference between **I** and **II** is in the ansa chain.

One ionizable function with pKa 4.9 can be spectrophotometrically detected for **I** in aqueous solution. By potentiometric titration in methylcellosolve - H_2O (4: 1), a pKa 6.1 is obtained. These observations indicate that the same acidic function exists on the chromophore of **I** as in **II**. Rifamycin Z is unstable in basic solution.

The IR spectrum of **I** in $CDCl_3$ solution as compared with that of **II**[1,3] has two additional bands [1730 ($\nu_{C=O}$) and 1250 ($\nu_{C\text{-}O\text{-}C}$) cm^{-1}] assignable to a lactone group. Also, the different absorption of the OH groups in the ansa chain, in particular $\nu_{OH}=3600\ cm^{-1}$, is related to a different hydrogen bonding, suggesting a different conformation of the ansa chain.

The ^1H NMR data of rifamycin Z (**I**) are reported in Table 1. When the data of **I** are compared with those of **II**[1], it is evident that the former lack the AB system at δ 3.78 and 4.08 ppm, due to CH_2OH-34a and the paramagnetic shift at H-25. Since all the other protons show a similar data, the main structural variations must occur at C-34a and C-25. The different J_{vic} values from H-23 to H-27 indicate that the conformation of the ansa chain in **I** is quite different from that in **II**, as may be expected from the lactonization of the COOH-34a with the OH-25. This difference in conformation is also reflected by the diamagnetic shift of nearly all the protons of **I** with respect to **II**; in fact, different aromatic solvent in-

Table 1. ^{1}H NMR data of rifamycin Z (**I**) at 270 MHz in pyridine-d_5, DMSO-d_6-acetone-d_6 (3: 1) and acetone-d_6, solutions, concentration $\sim 1.5 \times 10^{-2}$ M.

Proton	Multiplicity[a]	δ, ppm; TMS=0.00			
		Pyridine-d_5	DMSO-d_6-acetone-d_6 (3: 1)	Acetone-d_6[b]	J(Hz)[c]
H-3	s	7.77	7.30	7.52	—
OH-6	bs	7.7	10.5	—	—
OH-8	s	9.4	12.7	—	—
NHCO	s	n.d.	8.9	—	—
H-13	s	1.92	2.02	2.08	—
H-14	s	1.62	2.15	2.15	—
H-17	dq	6.01	6.4	6.34	17, 18=10
H-18	dd	6.64	6.4	6.46	18, 19=15
H-19	dd	5.58	5.78	5.79	19, 20=5.5
H-20	ddq	2.20	2.20	2.27	20, 21=9.5
H-21	dd	3.90	3.65	3.83	21, 22=1
OH-21	d	n.d.	5.68	—	OH, 21=4
H-22	ddq	n.d.	1.70	1.83	22, 23=1
H-23	dd	3.50	3.20	3.40	23, 24=10
OH-23	d	n.d.	5.55	—	OH, 23=6
H-24	ddq	n.d.	1.70	1.89	24, 25=2
H-25	dd	4.71	4.07	4.21	25, 26=11.5
H-26	ddq	n.d.	1.70	1.75	26, 27=4
H-27	dd	4.15	3.65	3.86	27, 28=1.5
OH-27	d	n.d.	4.34	—	OH, 27=1.5
H-28	dd	3.90	3.50	3.55	28, 29=8
H-29	d	6.64	6.20	6.30	
H-30	d	1.74	1.96	2.01	30, 17=0.5
H-31	d	0.63	0.74	0.84	31, 20=7
H-32	d	0.88	0.98	1.00	32, 22=7
H-33	d	0.58	0.68	0.77	33, 24=7
H-34	d	0.79	0.77	0.91	34, 26=7

a) Splitting of non-mobile protons with hydroxyl protons not considered.
b) Mobile protons exchanged with the solvent.
c) Coupling constants determined in acetone, except for hydroxyl protons, determined in DMSO-d_6-acetone-d_6=3: 1.
n.d.=Not determined.

duced shifts (ASIS) indicate different solute-solvent interactions. The ^{1}H NMR data of **I** in DMSO-d_6 - acetone-d_6 (3: 1) are also quite useful in assigning all the hydroxyl protons of the ansa chain (OH-21, 23 and 27) which had not been reported to date for the ansamycins.

The ^{13}C NMR data of **I** in DMSO solution are reported in Table 2. By comparing them with those previously reported for rifamycin W[1,4], a general similarity exists except for C-34a at δ 169.9 and C-25 at δ 78.7 ppm for rifamycin Z, as depicted in structure **I**. The six-membered lactone ring present in the ansa chain of rifamycin Z as the following configuration.

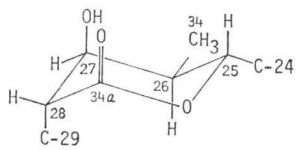
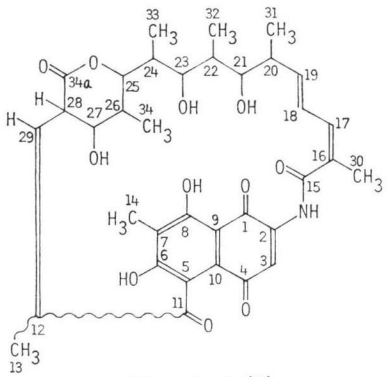
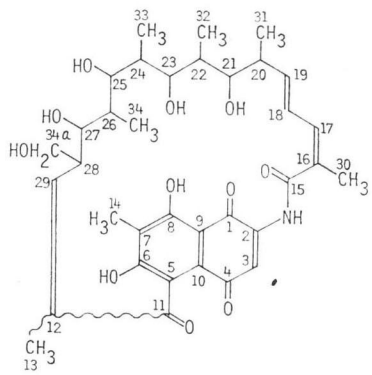


Table 2. ^{13}C NMR data of rifamycin Z (I) at 67.88 MHz in DMSO-d_6 solution, concentration 5×10^{-1} M, with TMS as internal reference.

Carbon	δ (ppm)	J ^{1}(Hz)	Carbon	δ (ppm)	J ^{1}(Hz)
1	181.8	—	18	124.2	155
2	140.4	—	19	139.9	150
3	114.2	175	20	38.0	130
4	184.0	—	21	71.7	140
5	123.5	—	22	32.2	125
6	160.0	—	23	76.8	140
7	117.6	—	24	35.1	125
8	162.1	—	25	78.7	145
9	106.5	—	26	38.8	130
10	127.7	—	27	77.2	150
11	195.5	—	28	51.6	140
12	141.7	—	29	133.2	160
13	19.1	125	30	11.8	125
14	8.7	125	31	15.2	125
15	171.6	—	32	11.6	125
16	132.3	—	33	8.7	125
17	131.9	155	34	16.4	125
			34a	169.9	125



Rifamycin Z (I)



Rifamycin W (II)

The absolute configuration of C-25, C-26 and C-27 is that reported for rifamycin B[5], while that of C-28 is assumed *R* according to the biogenetic pattern described by BRUFANI *et al.* for ansamycins[6]. It is quite interesting to point out that the relative configuration of the protons of the ring matches that deduceable from the vicinal coupling constants of the protons H-28, H-27, H-26 and H-25 (see Table 1). In fact, $J_{27,28}=1.5$, $J_{26,27}=4$ and $J_{25,26}=11.5$ correspond to a diequatorial, axial-equatorial and diaxial interactions, respectively, of the concerning protons.

Biosynthesis

We propose that rifamycin Z derives biosynthetically from rifamycin W by the oxidation of the hydroxymethyl group C-34a to a carboxyl which then lactonizes with the hydroxyl at C-25. The same relationship probably exists between streptovaricin G and F[7] and between protorifamycin I lactone and

protorifamycin I[8]. A biogenetic relationship between the known ansamycins has been recently reported by GHISALBA *et al.*[8].

In our opinion rifamycin Z has its counterpart in the protorifamycin I lactone, thus confirming the hypothesis that rifamycins and 8-deoxyansamycins follow two parallel biosynthetic pathways.

Biological Activity

Rifamycin Z shows no activity against Gram-positive bacteria, Gram-negative bacteria and *Mycobacterium tuberculosis*.

Experimental

UV-VIS spectra were measured on a Perkin-Elmer 4000 and IR spectra on a Perkin-Elmer 580 spectrometer. Mass spectra were obtained in direct inlet system at 70 eV on a Hitachi-Perkin Elmer RMU-6L spectrometer. ^{1}H NMR spectra and data at 270 MHz and ^{13}C NMR spectra and data at 67.88 MHz were determined on a Bruker WH-270 FT NMR cryospectrometer equipped with a 36 K BNC-12 computer and a disk unit. Solvents used were pyridine-d_5 and acetone-d_6 - DMSO-d_6 with TMS as the internal reference. Analytical TLC's were carried out on silica gel (Merck HF_{254}) plates using $CHCl_3$ - MeOH (9: 1) as the mobile phase.

Column chromatography was performed with 0.05~0.20 mm silica gel (Merck).

Occurrence and Isolation of Rifamycin Z

Mutant S/725 is a morphological variant of *N. mediterranea* strain ME/291 obtained by mutation of spores with nitrosoguanidine (conditions as in Ref. 9). Rifamycin Z was produced by fermentation of the above mutant in a complex organic medium[10] for 168 hours at 28°C. Fermentation broths were filtered, adjusted to pH 2.0 and extracted with ethyl acetate; the crude material obtained was purified by a countercurrent distribution of 180 transfers with benzene - methanol - 0.01 N HCl - hexane (3: 2: 1: 1) as solvent system. The appropriate fractions containing rifamycin Z were pooled, concentrated and purified by column chromatography, with $CHCl_3$ - MeOH (95: 5) as eluent. Yellow crystals of pure rifamycin Z were obtained from ethyl acetate, m.p. 253~255°C (dec.). Rf under the above condition was 0.32.

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