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STRUCTURE OF RIFAMYCIN W

A NOVEL ANSAMYCIN FROM A MUTANT OF *NOCARDIA MEDITERRANEI*

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Abstract—On the basis of spectroscopic studies (UV, IR, MS, ^1H and ^{13}C NMR) on rifamycin W and its tetrahydro- and dimethyl-derivatives, in comparison with the model compound rifamycin S (2), structure 1 is assigned to rifamycin W, a novel ansamycin isolated from a mutant strain of *Nocardia mediterranei*. The structural similarity of rifamycin W with other rifamycins and with streptovaricins is discussed from the biosynthetic point of view.

INTRODUCTION

The rifamycins are a family of ansamycin antibiotics[1,2] isolated from the fermentation medium of *Nocardia Mediterranei*. During our studies on mutant strains of this producer organism, a morphological variant (mutant 126) was isolated which produced a mixture of novel ansamycins. The major component of this complex, rifamycin W, has been isolated in a pure form and in the present paper we present evidence for its structure. The isolation, biosynthesis and metabolic role of this novel rifamycin are discussed in a separate publication.[3]

Elucidation of the structure

Rifamycin W (1) was obtained from ethyl acetate as yellow crystals. Elemental analysis and the molecular ion in the mass spectrum at *m/e* 655, supplemented by ^1H and ^{13}C NMR spectroscopy, account for the molecular formula $C_{35}H_{45}O_{11}N$.

The UV–VIS spectrum of 1 (Fig 1) suggested the presence of a chromophore somewhat different from that of rifamycin S (2).[4] In particular, there are two ionizable functions on the chromophore, evidenced by the variation of the UV–VIS spectrum in aqueous solution with pH ($pK_{a1} = 5{\cdot}0$, $pK_{a2} = 11$); the first of these functions was also revealed by potentiometric titration.

The polarographic behaviour, showing a two electron thermodynamically reversible reduction wave with $E_{1/2} = -0{\cdot}3$ volt *vs* S.C.E. (under the same conditions 2[5] shows $E_{1/2} = +0{\cdot}03$ volt) is in accordance with the presence of the 1,4-quinone-hydroquinone system.

The IR spectrum in nujol mull of 1 (Fig 2) is quite different from that of 2. It lacks the bands at 1725 cm^{-1} (ν C=O of dihydrofuranone carbonyl) and at 1700 cm^{-1} (ν C=O of acetoxyl group). The two bands at 1690 and 1630 cm^{-1}, also present in 2, are attributable to the amide carbonyl and to the quinone carbonyl linked in intramolecular H-bond; the band at 1490 cm^{-1} indicates that the chromophoric system is in the quinone form, on the basis of our observations in the rifamycin series.[6]

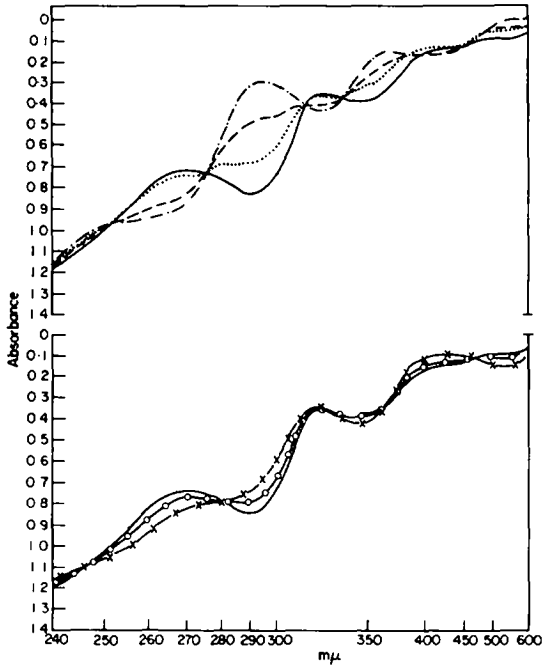


Fig 1. UV-VIS spectra of rifamycin W in aqueous solution at different pH values (conc. 0·0214 g/l); —·—·— pH = 1·0; ———— pH = 4·6;..... pH = 5·5; ———Ph = 7·4; —○—○—pH = 10·4; —×—×—pH = 11·5.

The data so far reported allow us to hypothesize the following partial structure for the rifamycin W chromophore, where the meta position of the two phenolic hydroxyls derives from analogy with 2.

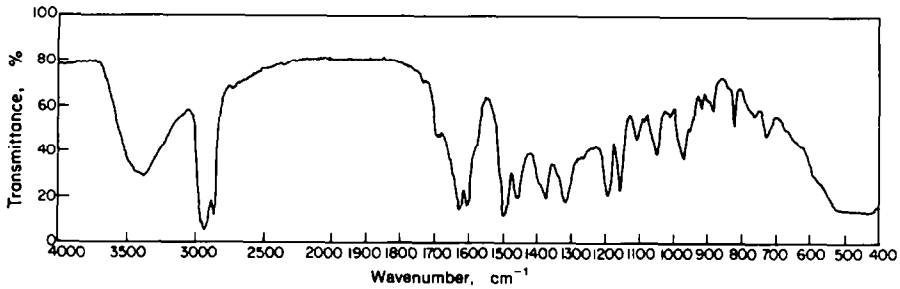
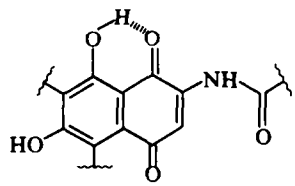
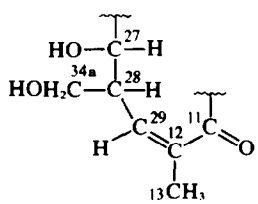


Fig 2. IR spectrum of rifamycin W in nujol mull.



The ^{1}H NMR spectrum of **1** in pyridine-d_5 is shown in Fig 3, where all the non-mobile protons are assigned. In particular, the sequence of protons in the ansa chain has been determined by ^{1}H homo-decoupling. The assignments, δ values, multiplicities and vicinal interproton coupling constants for the ansa protons of **1** are reported in Table I. The comparative examination of these data with those reported for **2**[7] allows us to postulate the same structure for **1** from C-17 to C-27, lacking the acetoxyl at C-25 and the methoxyl at C-27. Furthermore, as the J_{vic} values from H-17 to H-21 are the same in **1** and **2** the configuration of the two double bonds of the dienamide system must be the same. From C-27 to C-11 the following sequence is proposed for **1**:



on the basis of the following evidence:

(a) The complex multiplet at 3·12 ppm results from the coupling of H-28 with four protons; namely, the two non-equivalent protons of CH_2-34a at 3·78 and 4·08 ppm, H-29 at 7·18 ppm, and H-27 at 5·26 ppm. In other words, the protons of the moiety from C-27 to C-29 form a 5 spin system, which, by first order analysis, gives the parameters reported in Table I.

(b) The ppm value of H-29 is in good agreement with an olefinic proton β to a carbonyl function and this proton has an allylic coupling with CH_3-13 at 2·34 ppm.

The total number of mobile protons present, as determined by ^{1}H NMR and IR[8] is eight; in particular, the ^{1}H NMR spectrum in $CDCl_3$/DMSO-d_6 3/1, indicated the presence of the H-bonded phenolic OH on C-8 (sharp singlet at 12·15 ppm), of an acidic phenolic OH (broad band 8–10 ppm) attributable to the –OH at C-6, of the amide proton at 8·55 ppm and of five alcoholic protons (3·4–4·5 ppm) which can be assigned to be ansa chain hydroxyls on C-21, C-23, C-25, C-27 and C-34a. The presence of seven OH groups in the molecule was confirmed by the MS spectrum of the peracetylated derivative, obtained in microquantities, that had the molecular ion at *m/e* 949.

The MS spectrum of **1**, examined in comparison with that of **2**[9] does not show the [M-MeOH]$^+$ ion, characteristic of the presence of the C-27 methoxyl group and shows chromophoric ions at *m/e* 246, 273, 274 and 285, indicating structural differences in accordance with the absence of the dihydrofuranone ring in **1**.

From all the mentioned data it is possible to propose structure **1** for rifamycin W.

Support for structure **1** was obtained by the preparation of two derivatives. Treatment of **1** with methyl iodide yielded a product which proved to be 6,8-dimethyl rifamycin W on the basis of its mass spectrum, showing the molecular ion at *m/e* = 689 and the dimethyl chromophoric ions at *m/e* 274, 302 and 313, of the UV spectrum, which shows no variation in the pH range 2–11, and of the ^{1}H NMR spectrum, which is consistent with the presence of two aromatic –OMe groups. Catalytic reduction of **1**[4] afforded a substance which was recognized as 16, 17, 18, 19-tetrahydro rifamycin W on the basis of its ^{1}H NMR spectrum (DMSO-d_6), showing only one olefinic proton at 6·22 ppm (d, J = 8 Hz), while one of the singlets due to the Me groups on double bonds changed to a doublet (J = 7 Hz) at 1·20 ppm.

^{13}C NMR spectroscopy fully confirms structure **1**. In Fig 4 the FT ^{13}C proton-decoupled and in Fig 5 the non-decoupled spectra of **1** in DMSO-d_6 are reported. The assignments have been made by comparison with the ^{13}C NMR spectrum of **2** in $CDCl_3$[10,11] and DMSO-d_6 and with the off-resonance spectrum of **1**. Certain of these assignments played

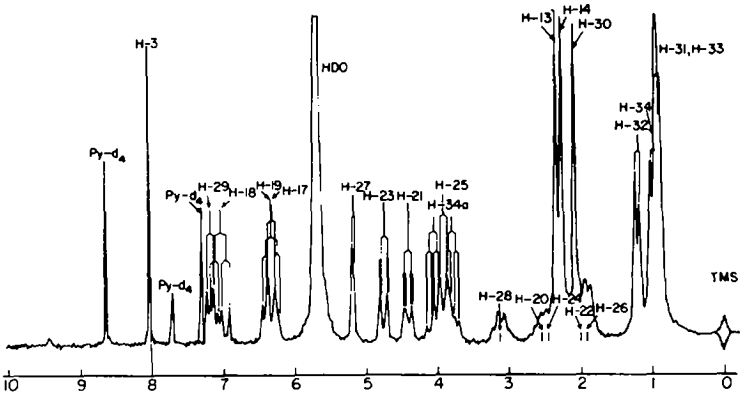


Fig 3. ^{1}H cw NMR spectrum of rifamycin W at 100 mHz in Py-d_5. (+ D_2O)

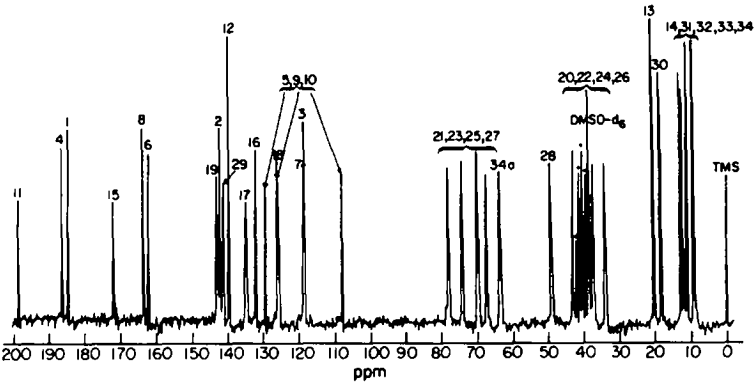


Fig 4. ^{13}C ft proton noise-decoupled spectrum of rifamycin W at 25·2 MHz in DMSO-d_6.

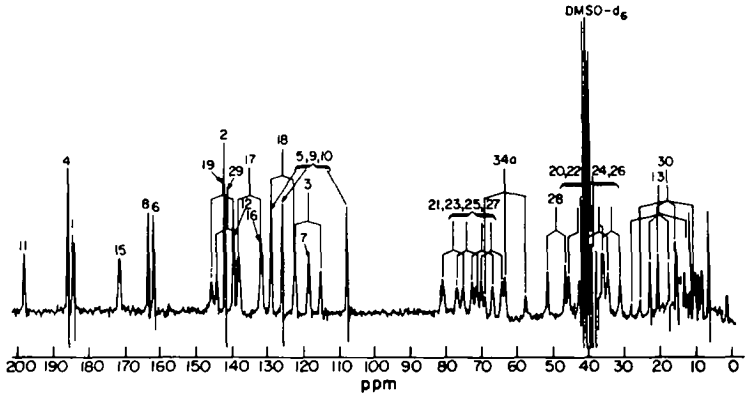
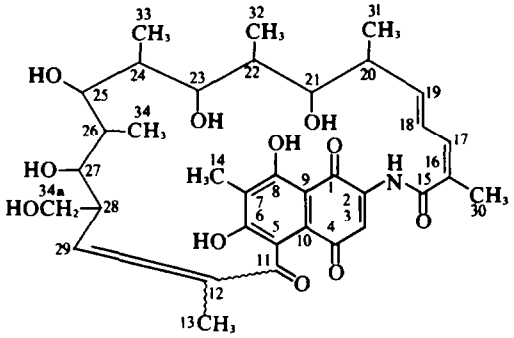


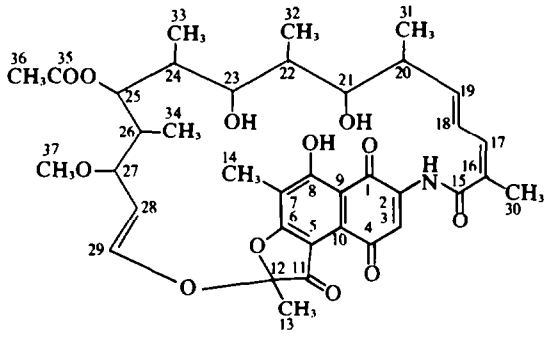
Fig 5. ^{13}C ft proton non-decoupled spectrum of rifamycin W at 25·2 MHz in DMSO-d_6.

a key role in confirming the proposed structure, in particular: (1) the presence of the new hydroxymethyl group C-34a at 63·1 ppm; (2) the shift of the methine group C-28 from 115·5 ppm (2, $CDCl_3$) to 48·9 ppm; (3) the upfield shift of C-6 with respect to the same carbon in 2; (4) the chemical shifts of C-11 and C-29, in agreement with the conjugation of the CO group with the double bond C-12 = C-29.[12]

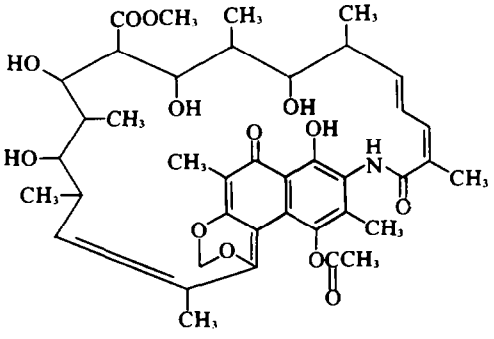
^{1}H NMR spectroscopy has also provided information on the conformation of the ansa chain of 1; this reasoning is made on the assumption that the various chiral centres of 1 and 2 have the same configuration, as they have the same biosynthetic origin.[3] Comparison of the data (see Table I) with



1 Rifamycin W



2 Rifamycin S



3 Streptovaricin D

Table 1. ^{1}H NMR data of rifamycin W in pyridine-d_5 (+ D_2O)

Proton	Multiplicity	δ (ppm, TMS = 0·00)	J (Hz)	Proton	Multiplicity	δ (ppm, TMS = 0·00)	J (Hz)
H-3	s	8·08	—	H-25	dd	3·94	$J_{25,26}$ = 1·0
H-13	s	2·34	—	H-26	m	2·00	$J_{26,27}$ = 1·5
H-14	s	2·28	—	H-27	dd	5·26	$J_{27,28}$ = 0·5
H-17	d	6·31	$J_{17,18}$ = 11·0	H-28	m	3·12	$J_{28,34a}$ = 7
H-18	dd	7·00	$J_{18,19}$ = 16·0	H-29	d	7·18	$J_{28,29}$ = 10
H-19	dd	6·32	$J_{19,20}$ = 6·5	H-30	s	2·08	—
H-20	m	2·61	$J_{20,21}$ = 9·0	H-31	d	0·96	$J_{31,20}$ = 7
H-21	dd	4·46	$J_{21,22}$ = 1·5	H-32	d	1·23	$J_{32,22}$ = 7
H-22	m	2·06	$J_{22,23}$ = 9·0	H-33	d	0·96	$J_{33,24}$ = 7
H-23	dd	4·81	$J_{23,24}$ = 1·0	H-34	d	1·03	$J_{34,26}$ = 7
H-24	m	2·54	$J_{24,25}$ = 10·0	H-34a	2dd	ν_1 = 3·78 ν_2 = 4·08	J_{gem} = 10

those for 2[7] makes the following conclusions possible: (1) the conformation of the H-17/H-18 bond is substantially the same, as reflected by the ppm values of H-17, H-18, H-19;[13] (2) the dihedral angles H-19/H-20 and H-20/H-21 are equal to those of 2, as shown by the J_{vic} values for these protons; (3) the conformation of all the bonds from C-21 to C-27 is quite different from that of 2 as indicated by the differing J_{vic} values of the respective protons and by the chemical shifts of the ansa chain Me groups which are no longer shielded in the same way (Me 34 is no longer above the plane of the chromophore as in 2[14]). No evidence is yet avaible for the configuration of the C-12/C-29 double bond and for the conformation of the C-5/C-11 bond.

Structural relationship of rifamycin W to other ansamycins.

Previous studies in our laboratories on the biosynthesis of rifamycin S using ^{13}C enriched precursors[15] suggested that this antibiotic derived from a progenitor having fundamentally the same carbon skeleton as the streptovaricins:[16] for example see streptovaricin D, formula 3, which lacks the

ether linkage interrupting the ansa chain of the rifamycins, and carries and extra carbon on C-28, which derives from C-3 of propionate.[17] The structure we now assign to rifamycin W includes both these features and in a separate publication[3] we show that this novel ansamycin derives from a single polyketide chain comprised of eight propionate and two acetate units, initiated by a seven carbon amino moiety in the same way proposed for rifamycin S.[15] Thus, rifamycin W represents the missing link in the biosynthetic pathway leading to the naphthalenic ansamycins.

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 and ^{13}C NMR spectra on a Varian XL-100 instrument equipped with Ft accessory. Mass spectra were recorded in DIS at 70 eV on a Hitachi-Perkin Elmer RMU-6L spectrometer. TLC analyses were carried out on silicagel Merck HF_{254} plates using $CHCl_3$/MeOH 7/3 as solvent system.

6,8-*Dimethylrifamycin W.* A soln of 500 mg of **1** in 4 ml MeOH was added to 10 ml $CHCl_3$, 7 g MeI and 750 mg Ag_2O and the mixture was refluxed for 1 hr. The mixture was filtered, taken to dryness, redissolved in $CHCl_3$ and chromatographed on a column of 50 g of silicagel (Merck 0·05–0·2 mm). The column was eluted with $CHCl_3$/MeOH 95/5 and 10 ml fractions collected. On TLC the major product was visible as a yellow spot with an R_f 0·5 in $CHCl_3$/MeOH 9/1. Fractions containing this product were pooled, taken to dryness, and 310 mg of yellow crystals were obtained from EtOAc.

16, 17, 18, 19-*Tetrahydrorifamycin W.* 600 mg of **1** were dissolved in 6 ml EtOH and 100 mg of hydrogenated Pt catalyst added. The mixture was then hydrogenated for 15 h at normal temp and pressure. The mixture was filtered, taken to dryness, redissolved in EtOAc and then extracted with 0·01 M sodium phosphate buffer pH 7·4. Reduced rifamycin W was recovered from buffer by acidifying and re-extracting with EtOAc, from which a crop of 200 mg of yellow crystals was obtained.

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