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THIAZO RIFAMYCINS—I

STRUCTURE AND SYNTHESIS OF RIFAMYCINS P, Q AND VERDE, NOVEL METABOLITES FROM MUTANTS OF *NOCARDIA MEDITERRANEA*

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Abstract—Structures 1, 2 and 3 have been assigned to rifamycins P, Q and Verde, novel metabolites isolated from a mutant strain of *Nocardia mediterranea* both on the basis of spectroscopic evidence (UV, IR, MS, ^1H and ^{13}C NMR) in comparison with the model compounds rifamycin S (4), rifampicin (5) and 4-dimethylamino-4-deoxy-rifamycin SV (6), and of an unambiguous synthesis from rifamycin S (4).

During our studies on mutant strains of *Nocardia mediterranea*, the producer organism of rifamycins,[1] three morfological variants, deposited with the American Type Culture Collection (ATCC 31064-31066) were isolated which produced a mixture of novel ansamycins.[2] The major components of this mixture, called rifamycins P, Q, R and Verde, were isolated in a pure form and in this paper we report the structure and synthesis of the new thiazo-rifamycins P, Q and Verde, while the structure of rifamycin R has been published elsewhere.[3]

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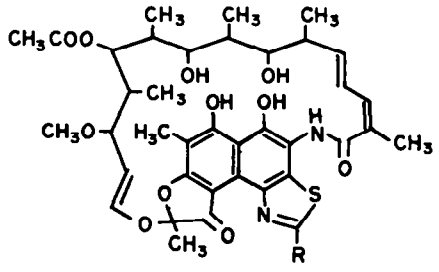
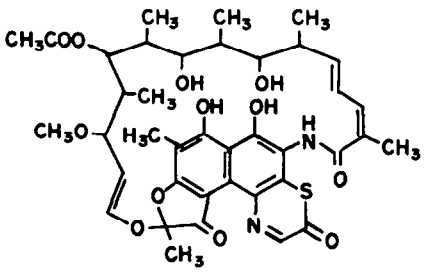
Structure determination

Rifamycins P(1) and Q(2) were obtained from ethyl acetate as yellow crystals. Rifamycin Verde (3) was obtained from ethylacetate-hexane as dark green powder. Evidence for the structure of these rifamycins was obtained by comparing their spectral data, MS, UV, IR, ^1H and ^{13}C NMR, with those of rifamycin S(4), rifampicin(5) and 4-dimethylamino-4-deoxy rifamycin SV(6),[4] used as model compounds (Fig. 1).

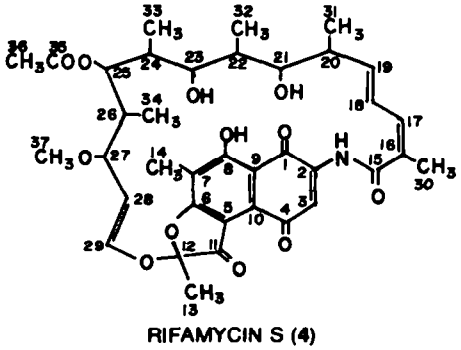
Rifamycin P(1). Elemental analysis and the molecular ion in the mass spectrum at m/z 738, supplemented by ^1H and ^{13}C NMR spectroscopy, account for the molecular formula $C_{38}H_{46}N_2O_{11}S$, i.e. 1 contains one C, H, N and S



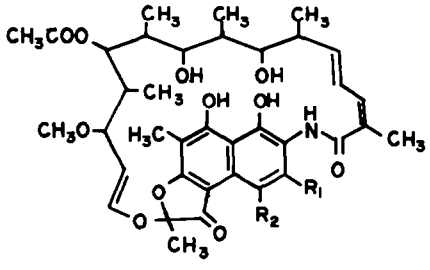
R = H, RIFAMYCIN P (1)
R = CH_2OH, RIFAMYCIN Q (2)



RIFAMYCIN VERDE (3)



RIFAMYCIN S (4)



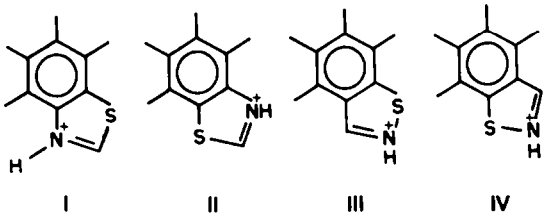
R_1 = CH=N-N N-CH, R_2 = OH — RIFAMPICIN (5)

R_1 = H, R_2 = $N(CH_3)_2$ — 4-dimethylamino 4-deoxy rifamycin SV (6)

Fig. 1. Structures of rifamycins P, Q, Verde, S, rifampicin and 4-dimethylamino 4-deoxy-rifamycin SV.

more and one O less than 4 ($C_{37}H_{45}NO_{12}$). The UV-visible spectrum of 1 (Table 1) shows that it contains a chromophore different from that of 4, 5 and 6. This is confirmed by comparing the mass spectra of 1 and 4; in fact, the same difference of 43 u between the molecular ions (m/z 738 vs 695) is observed also for the chromophoric ions (m/z 316 vs 273), while the pattern of fragmentation of the ansa chain is the same.[5] The variation of the electronic spectrum with pH and the potentiometric titration of 1, 2 and 3 indicate three ionisable functions, having the pKa_1, pKa_2 and pKa_3 values reported in Table 1. These three functions may be attributed to a strong acidic function (OH-8), to a basic function and to a weak acidic function (OH-1), respectively. The polarographic analysis does not reveal for 1, as well as for 2 and 3, the reversible redox wave (E 1/2 = 0.03 volts vs SCE for 4), thus indicating that the 1,4-quinone-hydroquinone system characteristic of rifamycins,[6] is blocked or absent. The IR spectrum of 1 in $CDCl_3$, examined in comparison with that of 4 and 6,[7] reveals two additional bands at 1420 and 1460 cm^{-1}, attributable to a benzothiazole ring.[8] These bands are shifted by deuteration meaning that the N of thiazole must be protonated.

The ^1H NMR data (Table 2) and ^{13}C NMR data (Table 3) show that the structure and conformation of the ansa chain of 1 from C-15 to C-12 is the same as that of 4,[9,10] 5 and 6, as reflected by the similarity of the ^1H and ^{13}C shifts and of the vicinal interproton coupling constants. The chromophore has the following features:(1) it is in the reduced form, because CH_3-34 and CH_3-33 (Table 2) are more shielded in the hydroquinone than in the quinone rifamycins,[11] (2) C-3 does not carry a proton [lack of the singlet at δ 7.87 in 4,[9] singlet at δ 136.4 (Table 3)]; (3) there are two mobile protons at δ 17.33 and 15.73, assigned, in analogy with 6, to OH-1 and $\overset{+}{N}H$= (Table 2); (4) 1, as well as 6 but not 5, exists in $CDCl_3$ solution, as an internal salt.[11] Thus, four structures are possible for the chromophore: two naphthothiazole (I, II) and two naphthoisothiazole (III, IV) types.



I II III IV

Types III and IV can be discarded because the singlet at δ 8.96 (Table 2) and also the doublet at δ 151.9 (Table 3) fits a benzothiazole ring better than a benzoisothiazole ring.[12] In deciding between I and II, the presence of the intramolecular H-bond between C_{11}=O and $\overset{+}{N}H$= in a 7-membered ring, as shown by the IR CO frequency at 1650 cm^{-1} in 6,[7] the CO ^{13}C NMR shifts at δ 187.7 in 1 and δ 185.2 in 6 (Table 3) are only consistent with the naphthothiazole structure I.

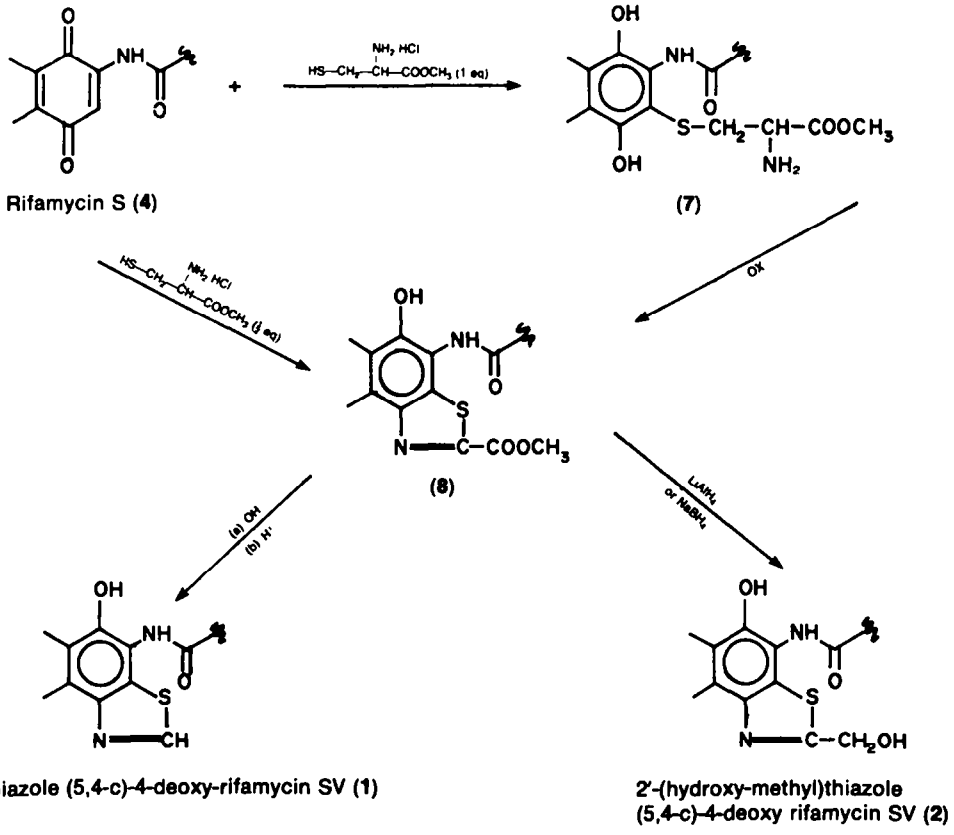
Rifamycin Q (2). A comparison of the physico-chemical data with those of 1, indicates that structure 2 (Fig. 1) can be assigned to rifamycin Q. Elemental analysis, mass spectrometry, ^1H and ^{13}C NMR account for the molecular formula $C_{39}H_{48}N_2O_{12}S$, i.e. 2 contains one C, O and two H more than 1. The UV-visible and IR spectra of 2 and 1 are almost superimposable. The ^1H NMR spectrum differs from that of 1 because it lacks the proton of the thiazole ring and shows the pattern of an additional ABX system, attributable to a $-CH_2OH$ group, analysed as reported in Table 2. This moiety is confirmed by the ^{13}C NMR spectrum of 2, which is identical to that of 1, except for C-2 of thiazole at δ 175.7 (singlet) and the additional triplet at δ 61.4 (Table 3).

Rifamycin verde (3). A comparison of the physico-chemical data with those of 1, indicates that structure 3 (Fig. 1) can be assigned to rifamycin Verde. Elemental analysis, ^1H and ^{13}C NMR account for the molecular formula $C_{39}H_{46}N_2O_{12}S$, i.e. 3 contains one C and O more than 1. The UV-visible spectrum shows that the chromophore of 3 is more conjugated than that of 1 (Table 1). The IR spectrum shows a trend similar to that of 1, except for the band at 1680 cm^{-1}, attributable to a thiolactone group.[13] The NMR data indicate that the difference between 3 and 1 resides in the condensed ring. The 2-oxothiazine moiety is assigned on the basis of the following evidence: (1) the proton at δ 7.71 coupled with the mobile proton at δ 17.03 (Table 2); (2) the tertiary carbon at δ 129.2, instead of δ 151.9 as in 1, and the new CO at δ 176.0 (Table 3); (3) also rifamycin Verde, as rifamycins P and Q, exists in $CDCl_3$ as an internal salt, with an intramolecular H-bond between C_{11}=O and the protonated nitrogen at C-4, and this excludes the isomeric structures with N at C-4; (4) the isothiazine structure with CO at C-3 can be discarded because in that case the ^1H and ^{13}C shifts of CH=N would be closer to those observed for 1.

Synthesis

The structures assigned to rifamycins P and Q have been confirmed by the synthesis outlined in Scheme 1. By reacting rifamycin S (4) with cysteine methyl ester hydrochloride, compound 7 is obtained in quantitative yield. The structure of 3 - [2 - amino - 2 - (methoxycarbonyl)ethyl] - thiorifamycin SV (7) derives from the negative Ellman reaction,[14] showing the absence of SH groups and the ^1H NMR spectrum (Experimental), showing the absence of H-3 and the presence of the three OH's at C-1,4 and 8, i.e. the cysteine methyl ester is linked through S to C-3. Similar S-substituted 3-thiorifamycins have been reported.[15] By treating compound 7 with various oxidative agents (Experimental), compound 8 is isolated in 20% yield. The same compound 8 can be obtained in 20% yield in one step by allowing rifamycin S (1 eq) to react in aqueous methanol with cysteine methyl ester hydrochloride (1/2 eq), thus using the same rifamycin S as an oxidatising agent.

The structure of 2' - carbomethoxythiazole(5,4-c) - 4 - deoxy rifamycin SV is assigned to compound 8, a key intermediate in the synthesis of rifamycins P and Q, on the basis of the following evidence: (1) the mass spectrum, showing the molecular ion at m/z 796; (2) the polarographic analysis, not showing the reversible redox wave of the 1,4-quinone-hydroquinone system; (3) the absence in the ^1H NMR spectrum of the $-CH_2-CH$ moiety and of the NH_2 group and the presence of two mobile protons at δ 16.53 and 15.86, assigned, in analogy with 6, to OH-1 and $\overset{+}{N}H$= and the OMe at δ 4.11 (Table 2); (4) the ^{13}C NMR data in comparison with those of 4[10] confirm structure 8: CO at δ 154.2, quaternary carbon at δ 159.1, OCH_3 at δ 53.8 (Table 3). Thus, the cyclisation of 7 gives rise to a thiazole ring instead of the expected

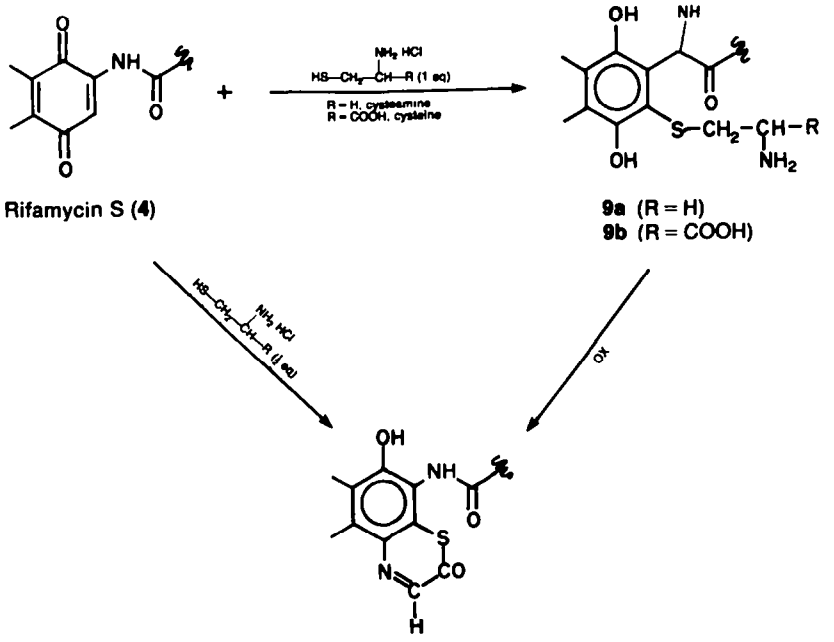


Scheme 1.

thiazine one; the same ring contraction occurs with the adduct of cysteine ethyl ester and *p*-benzoquinone.[16] Compound **8** gives thiazole (5,4-c)-4-deoxy-rifamycin SV (**1**) by alkaline hydrolysis and 2'-(hydroxymethyl)thiazole (5,4 - c) - 4 - deoxy - rifamycin SV (**2**) by reduction with LAH or $NaBH_4$ (Scheme 1). Compounds **1** and **2** so obtained are indistinguishable from natural rifamycins P and Q.

Rifamycin Verde has been obtained by the synthesis reported in Scheme 2.

By reacting rifamycin S (**4**) with cysteamine hydrochloride compound **9a**, already described,[15] is obtained.



Scheme 2.

This can be converted into 2'-oxo-thiazine (2,3-c)-4-deoxy-rifamycin SV (3) by means of various oxidising agents (Experimental). The same compound 3 can be obtained in one step by allowing one equivalent of 4 to react with half equivalent of cysteamine hydrochloride, thus using the excess of 4 as an oxidising agent. The same result is obtained through the open chain intermediate **9b**, prepared by reacting 4 with cysteine hydrochloride. In all cases compound 3 is obtained in a 60% yield and is indistinguishable from natural rifamycin Verde.

The oxidative cyclisation of **9a** to 3 forming the naphthothiazin-2-one is new and differs from that reported for 4 - methyl - *o* - benzo - quinone with cysteamine hydrochloride, which gives 5 - oxo - 7 - methyl - 8 - (2 - hydroxy - 4 - methylphenoxy) - 3,5 - dihydro - 2H - 1,4 - benzothiazine.[17] In a following paper a reaction mechanism for the ring contraction to naphthothiazole (Scheme 1) and the formation of the naphthothiazin-2-one (Scheme 2) will be proposed and accounted for.

EXPERIMENTAL

UV-VIS spectra were measured on a Perkin–Elmer 4000 and IR spectra on a Perkin–Elmer 580 spectrometer. ^{1}H NMR spectra at 60 MHz were obtained on a Bruker WP-60 FT NMR spectrometer, while ^{1}H NMR spectra and data at 270 MHz and ^{13}C NMR spectra and data at 67.88 MHz were obtained on a Bruker WH-270FT NMR cryospectrometer, equipped with 36 K BNC-12 computer and disk unit. The solvent used was $CDCl_3$ or DMSO-d_6 with TMS as the internal reference. Mass spectra were recorded in DIS at 70 eV on a Hitachi–Perkin–Elmer RMU-6L spectrometer. Analytical tlcs were carried out on silicagel Merck HF_{254} plates using $CHCl_3$/MeOH 9:1 as the mobile phase. Column chromatography was performed with 0.05–0.20 mm silicagel Merck.

Occurrence and isolation of rifamycins P, Q and Verde

The mutant strains are morphological variants of *N. mediterranea* strain ATCC 13685 obtained by mutagenesis of spores with nitroso guanidine (conditions as in Refs. 18, 19). The new rifamycins were produced by fermentation of the above mutants in a complex organic medium[20] for 200 hr at 28°. Fermentation broths were filtered, adjusted to pH 2.0 and extracted with EtOAc: rifamycins P, Q and Verde were extracted into 10 mM sodium phosphate buffer pH 7.38, while rifamycin R remained in the organic layer.[3] Acidification of the buffer soln and extraction with EtOAc gave the mixture of the three rifamycins which were separated by column chromatography on silicagel, eluting with $CHCl_3$ containing increasing amounts of MeOH. Rifamycin P was eluted with 2% MeOH, rifamycin Q with 3% and Verde with 5%, MeOH. The fractions containing these products were pooled, evaporated to dryness *in vacuo* and crystallised.

Representative yields from a 20 l. fermentor are: Rifamycin P: 150 mg (yellow crystals from EtOAc; m.p. > 190°, dec, R_f = 0.55); Rifamycin Q: 80 mg (yellow crystals from EtOAc; m.p. 179–80° dec; R_f = 0.4); Rifamycin Verde: 100 mg (dark green amorphous powder from EtOAc-hexane; m.p. > 200° dec; R_f = 0.3).

3 - [(2 - Amino - 2 - (methoxycarbonyl)ethyl - thio]rifamycin SV (7)

A soln of rifamycin S, (7 g; 0.01 m), cysteine methyl ester hydrochloride (1.7 g; 0.01 m) and NMe_3 (1.73 ml; 0.01 m) in 300 ml MeOH was heated under reflux. After 20 min the mixture was diluted with water (500 ml), acidified at pH = 2 and extracted with EtOAc (3 × 200 ml); the organic phase was dried (Na_2SO_4) and concentrated to about 20 ml. Compound 7 crystallised and after chilling, was collected and dried (6 g); dark-yellow crystals, m.p. > 160° dec; UV-VIS spectrum in buffer pH = 7.38: λ_{max} nm, (ϵ); 227 (37,750); 318 (21,600); 454 (13,300). ^{1}H NMR spectrum, (60 MHz, $CDCl_3$, δ): $-CH_2-CH$, m, 4.0–4.3 (3H); $-COOCH_3$, s, 3.78, (3H); NH_2,b, 5.1 (2H); OH-1,s, 13.00 (1H); OH-4.8, b, 15.8 (2H).

2' - Carbomethoxythiazole (5,4 - c) - 4 - deoxy - rifamycin SV (8)

(a) *By oxidation of 7 with rifamycin S.* A soln of 7 (3 g; 0.0036 m) and rifamycin S (6 g, 0.0085 m) in a mixture of MeOH (250 ml) and buffer (18 ml) pH = 4.6 was left at room temp for 16 hr. Tlc showed no more reactants and a new spot with R_f of about 0.4 and the spot due to rifamycin SV (R_f 0.05), besides trace amounts of by-products. The mixture was diluted with water (500 ml), acidified at pH = 2 and extracted with EtOAc (3 × 200 ml). A soln of 6 g of $K_3Fe(CN)_6$ in 500 ml of buffer pH = 7.38 was added to the organic phase and the mixture was stirred for a few min to oxidise the rifamycin SV to rifamycin S which in turn was extracted with EtOAc. The buffered soln was separated, acidified at pH = 2 and extracted with EtOAc. The organic phase was separated, washed with water, dried and concentrated to about 20 ml. Compound 8 crystallised and after chilling, was collected and dried (2 g); yellow crystals, m.p. 190–205° dec; UV-VIS spectrum in buffer pH = 7.38: λ_{max} nm, (ϵ); 225 (45,600); 295 (29,000); 394 (18,950).

(b) *By oxidation of compound 7 with 2,3 - dichloro - 5,6 dicyano - p - quinone.* Dichloro dicyano-*p*-quinone (230 mg; 0.001 m) was added to a soln of 7 (830 mg; 0.001 m) in 30 ml MeOH and 2 ml buffer pH = 4.6. After standing 15 hr at room temp the mixture was evaporated and the residue dissolved in $CHCl_3$. The organic phase was extracted with aqueous buffer pH 8.04, the buffered soln was separated, acidified at pH = 2 and then extracted with EtOAc. By concentration of the organic layer to small volume, 200 mg of 8 were obtained.

(c) *By oxidation of 7 with different oxidising agents.* The reaction was repeated employing equimolar amounts of other oxidising agents such as: tetrachloro-*p*-quinone, MnO_2, 2,5 -

Table 1. UV-visible data in phosphate buffer, pH = 7.38 and ionization constants of rifamycins P (1), Q (2), Verde (3), S(4), rifampicin (5) and 4-dimethylamino-4-deoxyrifamycin SV (6)

Rifamycins	λ_1(nm)	ϵ	λ_2(nm)	ϵ	λ_3(nm)	ϵ	λ_4(nm)	ϵ	λ_5(nm)	ϵ	pKa_1	pKa_2	pKa_3
1	260	31,100	297	22,900	405	14,000					<0	4.2	13
2	260	31,900	299	25,200	410	13,900					<0	4.2	13
3	223	33,000	328	11,500	372	11,500	480	7,600	720	6,900	<0	5.2	11.2
4[a]			317	29,600			525	4,300			6.7		
5[b]	237	33,200	255	32,100	334	27,000	475	15,400			1.7	6.7	
6[c]			302	18,800	418	22,000					0	10.8	

a) data from ref (21); b) data from ref (22); c) data from ref (4).

Table 2. ^{1}H NMR data of rifamycins P (1), Q (2), Verde (3) rifampicin (5), 4 - dimethylamino - 4 - deoxy - rifamycin SV (6) and 2' - carbomethoxythiazole (5,4 - c) - 4 - deoxy rifamycin SV (8) in $CDCl_3$ at 270 MHz, conc. 10^{-2} M[a]

PROTON	MULTIPL.	J (Hz) [b]	Chemical Shifts, δ (ppm)					
			1	2	3	5	6	8
OH-1	s	-	17.33	17.23	17.53	n.d.	17.90	16.53
NH	s [c]	-	15.73	16.16	17.03	n.d.	16.73	15.86
OH-4	s	-	-	-	-	n.d.	-	-
NH-CO	s	-	8.30	8.31	7.71	12.00	8.40	8.30
-CH=N-	s [c]	-/5	8.96	-	8.05	8.22	-	-
H-13	s	-	1.96	2.02	1.93	1.80	1.83	1.96
H-14	s	-	2.08	2.06	2.11	2.22	2.13	2.11
H-17	dq	17,18 = 10	6.28	n.d.	6.40	6.42	6.23	6.33
H-18	dd	18,19 = 15	6.48	n.d.	6.86	6.60	6.43	6.55
H-19	dd	19,20 = 7	6.24	n.d.	6.25	5.92	6.02	6.17
H-20	ddq	20,21 = 10	2.36	2.31	2.40	2.33	2.40	2.35
H-21	dd	21,22 = 2	3.54	3.46	3.70	3.78	3.70	3.58
OH-21	d	OH,21 = 1	3.52	3.53	3.45	3.43	3.66	3.55
H-22	ddq	22,23 = 2	1.66	1.63	1.66	1.72	1.75	1.70
H-23	dd	23,24 = 10.5	2.94	2.90	2.93	3.03	3.03	2.96
OH-23	d	OH,23 = 3.5	3.70	3.65	3.75	3.60	3.66	3.66
H-24	ddq	24,25 = 1	1.18	1.13	1.53	1.55	1.75	1.31
H-25	dd	25,26 = 10.5	4.83	4.71	4.90	4.93	4.92	4.84
H-26	ddq	26,27 = 1.5	1.12	0.96	1.16	1.37	1.40	1.26
H-27	dd	27,28 = 5	3.43	3.36	3.38	3.48	3.38	3.46
H-28	dd	28,29 = 13	5.06	4.95	4.93	5.10	5.25	5.16
H-29	d		6.06	6.01	5.93	6.20	6.18	6.15
H-30	d	30,17 = 0.5	2.14	2.16	2.15	2.07	2.08	2.11
H-31	d	31,20 = 7	0.84	0.81	0.96	0.88	0.85	0.85
H-32	d	32,22 = 7	0.97	0.95	0.98	1.02	1.05	0.98
H-33	d	33,24 = 7	0.36	0.25	0.48	0.60	0.57	0.38
H-34	d	34,26 = 7	-0.58	-0.78	-0.36	-0.30	-0.08	-0.50
H-36	s	-	2.00	2.00	1.96	2.05	2.00	2.01
H-37	s	-	3.04	3.00	3.00	3.05	3.10	3.08
CH_2-2',6'	m	n. d.	-	-	-	3.1	-	-
CH_2-3',5'	m	n. d.	-	-	-	2.5	-	-
NCH_3	s	-	-	-	-	2.33	-	-
$N(CH_3)_2$	2d	CH_3-$\overset{+}{N}H$ = 3.5	-	-	-	-	3.07 3.19	-
$-CH_2O$	2ddd	J_{AB} = 17	-	ν_A = 4.97 ν_B = 5.10	-	-	-	-
-OH	dd	OH, A = 5.5 OH, B = 8.5	-	5.33	-	-	-	-
$-OCH_3$	s	-	-	-	-	-	-	4.11
H-3	s	-	-	-	-	-	8.80	-

a) data for 5 and 6 refer to 10^{-3}M solutions, see ref. (11); b) average values of 5 and 6; 1,2,3 and 8 show quite similar values; c) d for 3.

Table 3. ^{13}C NMR data of rifamycins P (1), Q (2), Verde (3), rifampicin (5), 4 - dimethylamino - 4 - deoxyrifamycin SV (6) and 2' - carbomethoxythiazole (5,4-c) - 4 - deoxy - rifamycin SV (8) in $CDCl_3$ at 67.88 MHz, conc. ≈ 0.4 M. Chemical shifts, δ (ppm)

CARBON	1	2	3	5	6	8	CARBON	1	2	3	5	6	8
1	129.6	129.5	123.7	138.6	132.1	132.7	24	37.5	37.2	37.4	37.6	38.6	37.8
2	120.2	119.7	121.0	120.3	119.9	121.8	25	73.8	73.9	73.9	74.4	73.3	73.5
3	136.4	134.3	124.9	110.8	112.8	138.0	26	38.8	39.0	39.3	39.5	38.2	38.6
4	153.5	153.7	n.d.	147.8	155.1	154.0	27	77.6	77.2	77.1	76.7	80.5	78.2
5	113.0	112.4	117.9	112.8	113.2	113.6	28	117.3	117.2	117.9	118.7	115.8	117.2
6	174.1	172.1	173.6	174.3	173.3	171.9	29	141.8	141.9	141.9	142.6	143.8	142.0
7	106.4	106.4	106.1	105.9	103.6	107.2	30	20.2	20.0	20.2	20.7	20.3	20.2
8	168.2	168.7	167.6	169.3	169.5	168.1	31	18.0	18.1	18.2	17.8	17.5	17.7
9	100.0	99.6	101.8	104.4	97.7	100.4	32	11.0	10.9	10.9	10.9	11.3	11.0
10	116.3	114.8	116.5	117.8	123.8	117.2	33	8.6	8.4	8.9	8.5	10.5	8.5
11	187.7	186.6	192.5	195.3	185.2	188.4	34	8.7	8.7	9.1	8.8	8.8	9.1
12	110.9	110.9	108.6	108.7	109.5	111.2	35	172.0	170.8	171.8	171.9	172.0	172.0
13	21.5	21.4	20.7	21.5	22.0	21.9	36	20.7	20.7	20.7	20.7	20.9	20.7
14	7.0	7.0	7.0	7.6	6.8	7.1	37	57.2	57.2	57.4	57.0	56.8	57.2
15	169.5	170.0	170.0	169.6	169.5	169.1	-CH=N-	151.9	-	129.2	134.4	-	-
16	131.2	130.9	131.2	129.4	126.3	130.8	>C=N-	-	175.7	-	-	-	159.1
17	132.9	132.7	134.0	135.0	132.9	133.4	$-CH_2OH$	-	61.4	-	-	-	-
18	124.8	124.6	126.6	123.2	124.2	124.7	$-CO_2-(Me)$	-	-	-	-	-	154.2
19	141.1	141.5	140.4	142.6	141.1	141.4	-CO-S-	-	-	176.0	-	-	-
20	37.3	37.2	37.4	38.6	37.7	37.2	$-NCH_3$	-	-	-	45.8	-	-
21	73.8	74.0	74.4	70.7	72.3	73.7	$-N^{CH_3}_{CH_3}$	-	-	-	-	48.4 45.7	-
22	33.4	33.4	33.7	33.4	33.2	33.3							
23	76.7	76.9	76.6	76.7	77.0	77.1	-OMe	-	-	-	-	-	53.8

n.d. = not determined.

dimethyl - *p* - quinone, tetramethyl - *p* - quinone, 2,6 - dimethoxy - *p* - quinone and *p* - quinone under the same conditions as in (b). In each case the yields were of the same order as in (a) and (b).

(d) *From rifamycin S in one-step reaction.* A soln of 4 (7 g; 0.01 m) and cysteine methyl ester hydrochloride (0.85 g; 0.005 m) in 300 ml MeOH and 20 ml of buffer pH = 4.6 was left at room temp for 72 hr. By repeating exactly the work-up procedure described in (a), 2.4 g of 8 were obtained.

Thiazole (5,4-c)-4-deoxy-rifamycin SV (1)

A soln of 8 (2 g) in a mixture of 150 ml of acetone and 100 ml of 10% Na_2CO_3aq was stirred at room temp for 4 hr. The soln was acidified and extracted with EtOAc, the organic phase was separated, washed with water, dried (Na_2SO_4) and concentrated under vacuum to a small volume. 1 crystallised and after chilling, was collected and dried: 1.7 g.

The identity of 1 with rifamycin P obtained by fermentation was confirmed by physico-chemical analysis.

2' - (Hydroxy - methyl)thiazole (5,4 - c) - 4 - deoxy - rifamycin SV (2)

A soln of 8 (1 g) in 25 ml anhyd THF was added at room temp to a stirred suspension of LAH (0.5 g) in 10 ml anhyd THF. After 10 min, tlc showed no more 8 and a new spot with $R_f = 0.4$. The mixture was diluted with water, acidified, filtered and extracted with EtOAc. The organic phase was separated, washed with water, dried (Na_2SO_4) and concentrated to a small volume. Compound 2 crystallised and after chilling, was collected and dried (0.7 g). Compound 2 was obtained in about the same yield by employing 1 g of $NaBH_4$ instead of 0.5 g of LAH. The identity of 2 with rifamycin Q obtained by fermentation was confirmed by physico-chemical analysis.

3-[(2-Amino-2-carboxy)ethyl)thio]rifamycin SV (9b)

A soln of rifamycin S (7 g; 0.01 m) cysteine hydrochloride (1.57 g; 0.01 m) and (1.73 ml 0.01 m) NMe_3 in 300 ml MeOH was left at room temp for 2 hr and then concentrated to about 50 ml. Compound 9b crystallised and after chilling, was collected and dried (3 g); yellow crystals, m.p. 110°; UV-VIS spectrum in buffer pH = 7.38 λ_{max} nm, (ϵ); 266 (26,100); 315 (14,100); 430 (9,400). ^{1}H NMR spectrum (60 MHz, DMSO-d_6, δ): $-CH_2-CH$, m, 3.3-3.7, (2H); $-CH_2-\underline{CH}$ dd, 4.23, (1H); $\overset{+}{N}H_3$, b, 9.5, ($\overline{3H}$).

2' - Oxo - thiazine (2,3 - c) - 4 - deoxy - rifamycin SV (3)

(a) *By oxidation of 9a or 9b with rifamycin S.* A soln of 9 (0.004 m) (prepared according to Ref. 15) or of 9b and rifamycin S (0.004 m) in 300 ml MeOH was left at room temp for 16 hr, when tlc showed no more starting compounds, a new spot with R_f of about 0.3 and the spot due to rifamycin SV ($R_f = 0.05$) appeared besides trace amounts of by-products. The soln was concentrated to a small volume; compound 3 crystallised and after chilling, was collected and dried (2 g); yield 65%.

(b) *By oxidation of 9a with different oxidising agents.* The reaction was repeated employing equimolar amounts of the other oxidising agents used for the oxidation of 7–8. In each case the yields were of the same order as in (a).

(c) *From rifamycin S in one-step reaction.* A soln of rifamycin S (7 g; 0.01 m) and cysteamine hydrochloride (0.565 g; 0.005 m) or cysteine hydrochloride (0.775 g; 0.005 m) in 300 ml MeOH and 20 ml buffer pH = 4.6 was left at room temp for 24 hr. The soln was concentrated to about 80 ml; compound 3 crystallised and after chilling, was collected and dried (1.8 g).

The identity of 3 with rifamycin Verde obtained by fermentation was confirmed by physico-chemical analysis.

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