3-HYDROXYRIFAMYCIN S AND FURTHER NOVEL ANSAMYCINS FROM A RECOMBINANT STRAIN R-21 OF *NOCARDIA MEDITERRANEI*

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The structures of 3-hydroxyrifamycin S and six further novel ansamycins isolated from the recombinant strain R-21 of *Nocardia mediterranei* were identified by spectroscopic methods. Three types of structure were distinguished:

Type 1: Ansamycins of the rifamycin S type
Type 2: Ansamycins of the rifamycin G type
Type 3: Ansamycins of the rifamycin W type

As described in the foregoing paper the recombinant strain R-21 of *Nocardia mediterranei* elaborates a number of novel ansamycins[1]. Seven of these substances could be isolated and purified by repeated chromatography on silica gel columns and preparative TLC.

In this paper we report on the physicochemical properties and the structural elucidation of these novel ansamycins and give a brief account of their antimicrobial activity *in vitro*.

The seven rifamycins isolated can be assigned in three distinct classes:

1. Ansamycins of the rifamycin S type
   - 3-Hydroxyrifamycin S (**1**)
   - 3,31-Dihydroxyrifamycin S (**2**)
2. Ansamycins of the rifamycin G type
   - 16,17-Dehydrorifamycin G (**3**)
3. Ansamycins of the rifamycin W type
   - Rifamycin W-lactone (**4**)
   - Rifamycin W-hemiacetal (**5**)
   - 30-Hydroxyrifamycin W (**6**)
   - 28-Dehydroxymethyl-28,30-dihydroxyrifamycin W (**7**)

The structures of these novel ansamycins afford useful informations concerning the structure-activity relationships and the biosynthesis of the ansamycins.

Structures

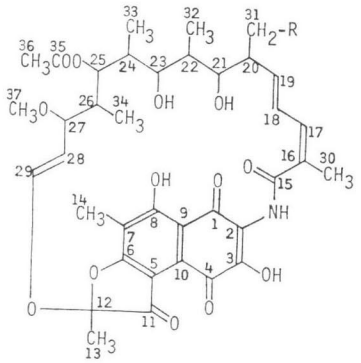
All the structures of the ansamycins from the recombinant strain R-21 have been elucidated by spectroscopic methods, in particular by direct comparison of their IR-, ^{13}C-NMR-, ^{1}H-NMR- and mass- spectra with the corresponding spectra of known rifamycins (*e.g.* rifamycins S[2,3], G[4] or W[5]).

3-Hydroxyrifamycin S (**1**) ($C_{37}H_{45}NO_{13}$)

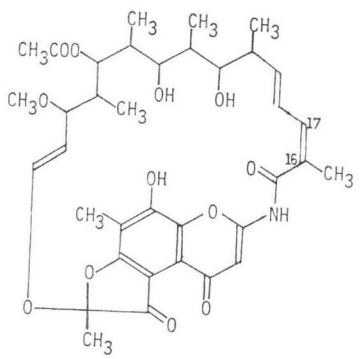
3-Hydroxyrifamycin S (**1**) which, apart from rifamycin B is the main product of fermentation of the R-21 strain forms reddish crystals from ether (m.p. 120~123°C; $[\alpha]_D = +700 \pm 1°$ (*c* 0.084, $CHCl_3$)). In the FD-mass spectrum the molecular ion was visible at *m/z* 711 which is 16 mass units higher than found for rifamycin S.* The ^{13}C-NMR- and the 360-MHz-^{1}H-NMR-spectra of **1** were compared with the corresponding spectrum of rifamycin S (*cf.* Tables 1 and 2 and Fig. 2). Whereas in the ^{13}C-NMR-

* The molecular ion M^+ of *m/z* 999 in the persilylated product with 4 silyl groups corresponds again to a molecular weight of 711.

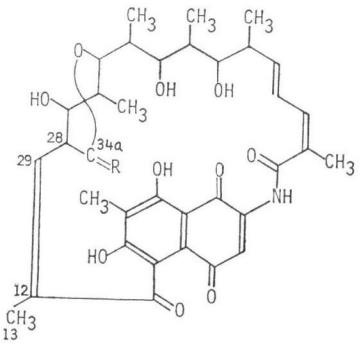
Fig. 1. Rifamycins from the recombinant strain R-21.



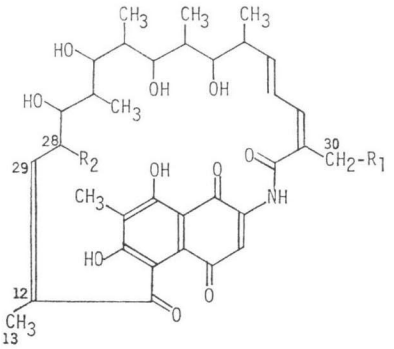
R=H: 3-Hydroxyrifamycin S (**1**)
R=OH: 3,31-Dihydroxyrifamycin S (**2**)



16,17-Dehydrorifamycin G (**3**)

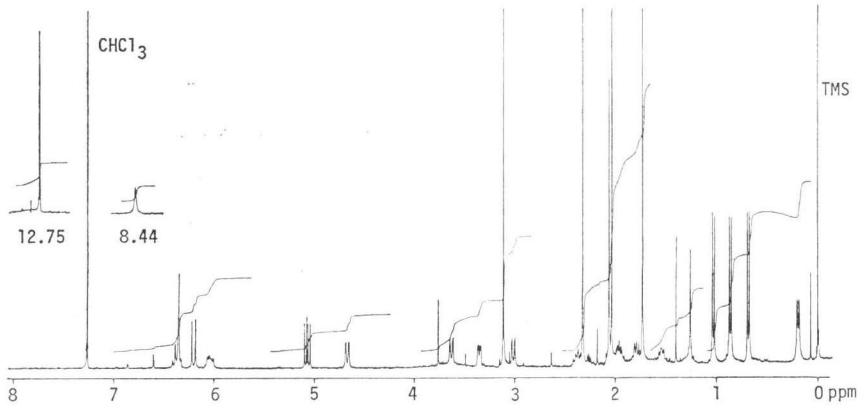


R=O: Rifamycin W-lactone (**4**)
R=H,OH: Rifamycin W-hemiacetal (**5**)



R_1=OH, R_2=CH_2OH: 30-Hydroxyrifamycin W (**6**)
R_1=OH, R_2=OH: 28-Dehydroxymethyl-28,30-dihydroxyrifamycin W (**7**)
R_1=H, R_2=CH_2OH: Rifamycin W

Fig. 2. 360-MHz-^{1}H-NMR-spectrum of 3-hydroxyrifamycin S ($CDCl_3$).



spectrum of **1** the carbon atoms of the ansa chain showed almost identical chemical shifts compared to rifamycin S, strong shift differences were observed for some C-atoms of the chromophore, due to the hydroxyl group in position 3. The most drastic shift differences were found for the C-atoms C-2, C-3 and C-4 which were localized at 118.4 ppm, 146.8 ppm and 177.2 ppm in **1** compared to 139.4 ppm,

117.4 ppm and 181.6 ppm, respectively, in rifamycin S.

In the 360-MHz-^{1}H-NMR-spectrum of **1** (see Fig. 2 and Table 2) the signal of the H-3 proton, normally present at 8.3 ppm in unsubstituted rifamycins, is missing. For the rest of the protons no significant shift differences were observed between **1** and rifamycin S.

Table 1. ^{13}C-NMR data of rifamycins S and G and the ansamycins from recombinant strain R-21 (δ values in ppm).

C	Rifa S ($CDCl_3$)	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	Rifa G (CD_3OD)	**4** (*d*-DMSO)	**5** (CD_3OD)	**6** (CD_3OD)	**7** (*d*-DMSO)
1	184.5	184.9	185.0	missing	missing	182.1	184.7	185.1	182.5
2	139.4	118.4	118.3	171.6	172.3	140.4*	139.5	146.2	140.5
3	117.4	146.8	147.1	99.2	98.7	117.8	117.4	116.7	115.1
4	181.6	177.2	177.3	178.8	179.8	184.2	186.9	187.3	183.8
5	111.2	111.0	111.0	109.1	109.0	123.8	118.5	119.9	123.4
6	172.2	171.9	172.0	155.9	157.4	160.2	161.8	161.8	160.3
7	115.7	116.8	116.7	115.4	114.3	114.4	~118	118.6	116.8
8	166.5	166.4	166.5	156.8	161.0	162.4	163.4	163.5	161.6
9	111.0	109.8	109.7	143.5	145.5	106.5	108.1	108.2	106.3
10	131.3	129.1	129.1	120.0	120.4	132.2	134.7	130.7	127.4
11	191.1	191.8	192.0	194.1	193.7	195.8	199.2	199.5	195.8
12	108.6	108.1	107.9	106.4	104.6	139.7	143.1	142.9	136.5
13	22.4	22.0	21.8	22.2	22.1	16.4	12.5	19.6	17.2
14	7.4	7.6	7.7	8.5	8.5	8.8	8.4	8.5	8.7
15	169.0	171.4	171.2	169.8	175.3	171.7	170.2	170.3	169.2
16	131.0	128.8	129.4	131.4	34.4	132.2	128.9	131.4	130.5
17	133.2	135.3	135.0	134.7	44.1	133.5	139.5	144.2	137.4
18	124.4	123.9	126.9	125.3	129.2	124.4	128.5	128.8	124.6
19	142.4	143.0	138.2	142.6	133.6	141.8	144.1	142.6*	141.0
20	39.2	38.8	46.5	40.2	41.5	39.8	42.6	43.0	37.4
21	73.6	73.2*	69.2	74.0	73.2*	71.6	72.8	73.5*	71.1
22	33.0	32.8	32.9	34.0	34.4	32.2	33.9	33.9	28.9
23	77.7	77.4	77.5	77.7	78.3	78.6	73.5	77.8	76.2
24	37.4	37.6	37.8	38.9	36.6	35.2	41.1	41.2	36.0
25	73.6	73.5*	73.6	74.0	75.3*	80.1	81.4	73.0*	74.5
26	37.4	37.4	37.6	38.4	38.9	41.5	46.4	47.2	n.l.
27	81.9	81.3	80.6	82.7	78.3	78.6	77.5	81.6	77.7
28	115.7	115.6	116.5	118.3	121.1	51.7	49.1	49.2	62.1
29	145.3	144.7	144.0	146.1	144.6	139.7	142.4	141.8*	138.7
30	20.0	20.1	20.1	20.2	15.6	19.4	19.5	94.1	83.1
31	16.8	16.9	62.3	17.3	17.3	15.4	12.3	11.6	14.7
32	11.4	11.6*	11.8*	12.1*	11.6*	11.9*	11.6*	12.4*	13.6*
33	8.8	8.9	8.7	9.4	8.9	8.8	11.5	11.6	11.1
34	11.4	11.3*	11.1*	11.4*	9.4*	11.7*	11.5*	12.6*	11.4*
35	172.6	173.0	173.0	172.9	172.7	—	—	—	—
36	20.9	21.0	20.9	21.1	20.9	—	—	—	—
37	56.8	56.8	56.9	56.5	56.9	—	—	—	—
34a	—	—	—	—	—	169.7	94.0	62.5	—

n.l.: Signal present but not localized.

* : Tentative assignment.

Further data: Rifamycin W see reference 15.

Table 2. ^{1}H-NMR data of the ansamycins from recombinant strain R-21.
(δ values in ppm, coupling constants J(Hz) in brackets)

Protons	3-Hydroxy-rifamycin S (**1**) ($CDCl_3$)	3,31-Dihydroxy-rifamycin S (**2**) ($CDCl_3$)	16,17-Dehydro-rifamycin G (**3**) ($CDCl_3$)	Rifamycin W-lactone (**4**) ($CDCl_3$)	Rifamycin W-hemiacetal (**5**) (*d*-DMSO)	30-Hydroxy-rifamycin W (**6**) ($CDCl_3$+*d*-DMSO)	28-Dehydroxy-methyl-28,30-di-hydroxyrifamycin W (**7**) (*d*-DMSO)
H (3)	not present	not present	7.18 (s)	7.44 (s)	7.63 (s)	7.5 (s)	7.38 (s)
H (8)	12.75 (s)	12.7 (s)	n.l.	12.00 (s)	12.15 (s)	12.04 (s)	12.25 (s)
H (17)	6.19 (d, 12)	6.33 (d, 12)	6.32 (d, 12)	6.31 (d, 9)	6.17 (d, 9)	6.5 (d, 12)	6.40 (d, 9)*
H (18)	6.38 (dd, 12, 16)	6.47 (dd, 12, 16)	6.40 (dd, 12, 16)	6.51 (dd, 9, 12)	6.67 (dd, 9, 16)	7.3 (dd, 12, 16)	6.20 (dd, 9, 16)*
H (19)	6.04 (dd, 9, 16)	5.95 (dd, 9, 16)*	5.83 (dd, 9, 16)	5.87 (dd, 6, 12)	5.80 (dd, 9, 16)	6.02 (dd, 9, 16)	5.80 (dd, 9, 16)*
H (28)	5.08 (dd, 6, 12)	5.00 (dd, 9, 12)	5.24 (dd, 9, 14)	n.l.	n.l.	n.l.	4.55 (dd, 6, 9)*
H (29)	6.38 (d, 12)	6.11 (d, 12)	6.25 (d, 14)	5.87 (d, 9)	5.75 (d, 9)	5.74 (d, 9)*	5.80 (d, 9)*
H (25)	4.68 (d, 9)	4.63 (d, 9)	4.68 (d, 9)	4.27 (d, 9)	3.77 (d, 9)	n.l.	n.l.
CH_3 (13)	1.74 (s)	1.67 (s)	1.72 (s)	2.00 (s)	1.87 (s)	1.93 (s)*	1.92 (s)*
CH_3 (14)	2.33 (s)	2.25 (s)	2.26 (s)	2.12 (s)	2.15 (s)	2.12 (s)	2.17 (s)
CH_3 (30)	2.08 (s)	2.00 (s)	1.96 (s)	2.04 (s)	2.08 (s)	not present	not present
CH_3 (31)	0.86 (d, 6)	not present	0.82 (d, 6)	0.98 (d, 6)	0.92 (d, 6)	1.02 (d, 6)	0.88 (d, 6)
CH_3 (32)	1.03 (d, 6)	1.00 (d, 6)	0.98 (d, 6)	1.12 (d, 6)	1.03 (d, 6)	1.15 (d, 6)	0.98 (d, 6)
CH_3 (33)	0.68 (d, 6)	0.63 (d, 6)	0.65 (d, 6)	0.89 (d, 6)	0.72 (d, 6)	0.83 (d, 6)	0.77 (d, 6)
CH_3 (34)	0.19 (d, 6)	0.1 (d, 6)	n.l.	0.78 (d, 6)	0.52 (d, 6)	0.69 (d, 6)	0.47 (d, 6)
H (20)	n.l.	2.45 (m)*	n.l.	n.l.	n.l.	n.l.	n.l.
H (21)	3.64 (d, 9)	3.95 (d, 9)*	n.l.	n.l.	n.l.	n.l.	n.l.
H (34a)	—	—	—	not present	4.87 (d)	n.l.	not present
CH_2OH (30)	—	—	—	—	—	*ca.* 4.3 (A, B)	4.18 (A, B)*
CH_2OH (31)	—	3.50 (A, B) *	—	—	—	—	—
OCH_3 (37)	3.12 (s)	3.04 (s)	3.1 (s)	—	—	—	—
$COCH_3$	2.05 (s)	1.96 (s)	1.96 (s)	—	—	—	—

n.l.: Signal present but not localized.

* : Position confirmed by double resonance experiment.

Further data: Rifamycin S see references 2 and 3, rifamycin G see reference 4 and rifamycin W see reference 5.

3,31-Dihydroxyrifamycin S (**2**)

In 3,31-dihydroxyrifamycin S (**2**), in addition to the hydroxyl group in position 3, the C-31 methyl group is also hydroxylated. The postulated structure, especially with regard to the position of the additional hydroxyl group at C-31, is based on the following observations:

In the EI-mass spectrum of the persilylated compound **2**, the molecular ion M^+ was found at *m/z* 1159 with six silyl groups, corresponding therefore to a molecular weight of 727 ($C_{37}H_{45}NO_{14}$).

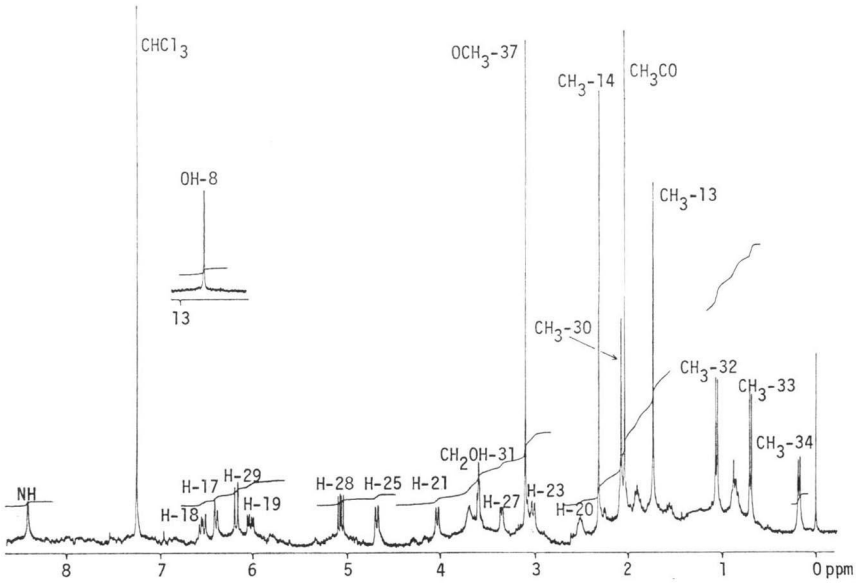
In the ^{13}C-NMR-spectrum of **2** (*cf.* Table 1), the signal of the C-31 methyl group, normally found at 16.8 ppm in rifamycin S (or 16.9 ppm in 3-hydroxyrifamycin S), is missing, whereas an additional signal of an oxygen substituted carbon atom was found at 62.3 ppm which can be attributed to the C-31 hydroxymethyl group. Due to this group C-20 shows a downfield shift and C-19 a small upfield shift (C-20: 46.5 ppm in **3** compared to 39.2 ppm in rifamycin S; C-19: 138.2 ppm in **2** compared to 142.4 ppm in rifamycin S). In the aromatic part of the molecule the chemical shifts were identical to those found in 3-hydroxyrifamycin S.

In the 360-MHz-^{1}H-NMR-spectrum of **2**, (*cf.* Table2 and Fig. 3) besides the signal of the H-3 proton, the doublet of the C-31 methyl group, normally localized at 0.85 ppm in rifamycin S is also missing. Instead of these, there is a multiplet at 3.50 ppm decoupling to an AB-system by irradiation at H-20 (2.45 ppm) which is attributed to the C-31 hydroxymethyl group. Furthermore, besides the coupling of the H-20 proton with this hydroxymethyl group, the interaction of this proton with the protons H-19 (5.95 ppm) and H-21 (3.95 ppm) could be demonstrated by decoupling experiments, thus proving the position of the hydroxyl group at C-31.

16,17-Dehydrorifamycin G (**3**)

16,17-Dehydrorifamycin G (**3**) is, after rifamycin G[4], the second example in the rifamycin series where C-1 is replaced by an oxygen atom, and a γ-pyrone ring is therefore present instead of the quinone of rifamycin S. The only structural difference between **3** and rifamycin G is that **3** possesses the intact ansa ring system of the rifamycins, whereas in rifamycin G the C-16–17 double bond is saturated. The ^{13}C-NMR-, ^{1}H-NMR- and mass-spectra of these two compounds can therefore be directly compared. In fact, in the EI-mass-spectrum there is a difference of only two mass units in the localisation of the molecular ion between **3** (M^+ at *m/z* 683, $C_{36}H_{45}NO_{12}$) and rifamycin G (M^+ at *m/z* 685, $C_{36}H_{47}NO_{12}$). Furthermore, except for the signals of C-16 and C-17, present as olefinic carbons at 131.4 ppm and 134.7 ppm in **3** but at 34.3 ppm and 44.1 ppm in rifamycin G, there is no substantial difference in the chemical

Fig. 3. 360-MHz-^{1}H-NMR-spectrum of 3,31-dihydroxyrifamycin S ($CDCl_3$).



shifts of the C-atoms in the ^{13}C-NMR-spectra of the two compounds (*cf.* Table 1).

The ^{1}H-NMR-spectrum of **3** (*cf.* Table 2) is in good agreement with that of rifamycin G previously reportet by the Lepetit group[4] from which it was evident that owing to the loss of the carbonyl group in position 1 the hydroxyl group in position 8 of the chromophore is no longer able to form the strong hydrogen bond indicated by a signal at 12.5 ppm in rifamycins with an intact chromophore. This signal is missing in **3** as well as in rifamycin G. Furthermore, the signal of the H-3 proton had shifted from 7.77 ppm in rifamycin S to 7.18 ppm in 16,17-dehydrorifamycin G (7.35 ppm in rifamycin G[4]) suggesting that this proton is no longer in β-position to a carbonyl group.

Rifamycin W-lactone (**4**)

In compound **4**, the C-34a hydroxymethyl group of rifamycin W[5] is oxidized to a carboxylic group, followed by lactone formation with the C-25 hydroxyl group. An analogous lactone was already observed in protorifamycin I-lactone, a metabolite recently isolated in our laboratories from the *Nocardia mediterranei* mutant F 1/24[5]. Protorifamycin I-lactone differs from **4** only in the absence of the hydroxyl group in position 8 of the chromophore; the ansa chain being unchanged in both compounds.

In fact, the molecular ion M^+ at *m/z* 651 in the FD-mass spectrum of **4** ($C_{35}H_{41}NO_{11}$) is 16 mass units higher than in protorifamycin I-lactone.

In the ^{13}C-NMR- and the ^{1}H-NMR-spectrum of **4**, (*cf.* Tables 1 and 2) the carbon and hydrogen signals of the ansa chain are substantially unchanged compared with these of protorifamycin I-lactone. Concerning the C-34a carbon atom it was found as a carbonyl signal at 169.7 ppm (174.4 ppm in protorifamycin I-lactone) in the ^{13}C-NMR-spectrum instead of 64.2 ppm in rifamycin W with a C-34a hydroxymethyl group. Consequently, the AB-system of the C-34a hydroxymethyl group normally present it 3.2 ppm in rifamycin W (or protorifamycin I) is missing in the ^{1}H-NMR-spectrum of **4**.

Rifamycin W-hemiacetal (**5**)

In the hemiacetal **5** the C-34a hydroxymethyl group of rifamycin W[5] is oxidized to the aldehyde followed by hemiacetal formation with the hydroxyl group in position 25 of the ansa chain. Compared to rifamycin W-lactone (**4**) the following differences in the spectra are evident: a) The FD-mass spectrum of **5** shows a molecular ion M^+ at *m/z* 653 ($C_{35}H_{43}NO_{11}$) which is 2 mass units more than found for the lactone **4**. b) In the ^{13}C-NMR-spectrum of **5** (*cf.* Table 2) the signal of C-34a is localized at 94.0 ppm compared to 169. 7ppm in the lactone, thus indicating the presence of an acetalic carbon. The remaining C-atoms of the molecule show no notable shift differences. c) In the 360-MHz-^{1}H-NMR-spectrum of **5** the acetalic H-34a proton is found at 4.87 ppm.

30-Hydroxyrifamycin W (**6**) ($C_{35}H_{41}NO_{12}$, FD-MS: M^+ at *m/z* 671)

The structure of **6** differs from rifamycin W[5] only by its primary hydroxyl group at C-30 as evidenced by lacking the signal of the C-30 vinylic methyl group found at 20 ppm in the ^{13}C-NMR-spectrum of rifamycin W. An additional signal of an oxygen substituted carbon at 94.1 ppm is now attributed to the C-30 hydroxymethyl group.

In the 360-MHz-^{1}H-NMR-spectrum only one vinylic methyl group is present (1.93 ppm). Its long range coupling with the olefinic H-29 proton could be demonstrated by a decoupling experiment proving therefore that the remaining vinylic methyl group has to be the C-13 one.

28-Dehydroxymethyl-28,30-dihydroxyrifamycin W (**7**)

The structure assignment of **7** is based on the following observations:

The molecular ion M^+ of *m/z* 657 in the FD-mass spectrum corresponds to a molecular formula of $C_{34}H_{43}NO_{12}$.

In the ^{13}C-NMR-spectrum (*cf.* Table 1) two additional signals of oxygen substituted carbons were found at 62.1 ppm and 83.1 ppm while the signals of the vinylic C-30 methyl group and C-34a, normally present at 20 ppm and 49 ppm, respectively in rifamycin W, are missing. The signal at 83.1 ppm is now attributed to the C-30 hydroxymethyl group and the one at 62.1 ppm to C-28 bearing a hydroxyl group.

The 360-MHz-^{1}H-NMR-spectrum (*cf.* Table 2) is in good agreement with the postulated structure of **7**. The signals of the vinylic CH_3 (C-30) protons as well as those of the C-34a hydroxymethyl group in position 28 are missing. A new AB-system at 4.18 ppm is attributed to the C-30 hydroxymethyl

group as could be demonstrated by an irradiation experiment. The H-28 proton bearing now a hydroxyl group instead of a hydroxymethyl group shifted downfield to 4.55 ppm. Its exact position, especially the coupling with the olefinic H-29 proton was confirmed by double resonance experiments.

Antimicrobial Activity

The *in vitro* minimal inhibitory concentration (MIC's in mcg/ml) against various strains of Gram-positive and Gram-negative organisms were determined by the two-fold agar dilution method on DST agar (Oxoid), with an inoculum of 10^4 organisms, deposited on the surface of the agar by means of a multiple replicating device[7].

The MIC-values of the biologically active ansamycins of the recombinant strain R-21 are shown in Table 3. The ansamycins of the rifamycin W type (rifamycin W-lactone, rifamycin W-hemiacetal, 30-hydroxyrifamycin W and 28-dehydroxymethyl-28,30-dihydroxyrifamycin W) are devoid of any biological activity and are therefore not included in Table 3.

Surprisingly, despite the replacement of the quinone ring by a γ-pyrone ring, 16,17-dehydrorifamycin G (**3**) still shows activity against Gram-positive bacteria. By contrast, rifamycin G with a saturated 16,17-double bond was reported to be inactive[4]. Furthermore 16,17-dehydrorifamycin G shows activity on the RNA-polymerase which is the target enzyme of the rifamycins, whereas rifamycin G is not active on the enzyme.* These results indicate that the presence of the 16,17-double bond is essential for biological activity of the rifamycins.

The most active compound from the recombinant strain R-21 is 3-hydroxyrifamycin S (**1**). Compared to rifamycin S **1** shows slightly improved activity against Gram-negative bacteria. 3,31-Dihydroxyrifamycin S (**2**), on the other hand is less active than rifamycin S thus indicating that alterations in the structure of the ansa chain are critical, and in most cases, give less active compounds.

Table 3. Antibacterial spectrum of 3-hydroxyrifamycin S, 3,31-dihydroxyrifamycin S and 16,17-dehydrorifamycin G in comparison to rifamycin S (MIC values in mcg/ml).

Organism	3-Hydroxy-rifamycin S	3,31-Dihydroxy-rifamycin S	16,17-Dehydro-rifamycin G	Rifamycin S
Staphylococcus aureus 10 B	0.1	2	1	0.01
Staphylococcus aureus 2999	0.1	4	1	0.01
Streptococcus pyogenes Aronson	0.1	1	1	0.01
Streptococcus faecalis 1362/3	64	>128	128	16
Neisseria gonorrhoeae 1317/4	0.1	0.1	0.5	0.1
Haemophilus influenzae NCTC 4560	1	2	4	0.01
Escherichia coli 205	32	>128	>128	128
Escherichia coli 1074	64	>128	>128	>128
Klebsiella pneumoniae 327	64	>128	>128	>128
Enterobacter cloacae P99	64	>128	>128	>128
Proteus mirabilis 774	16	>128	>128	64
Proteus rettgeri 856	64	>128	>128	>128
Proteus morganii	32	128	>128	64
Pseudomonas aeruginosa ATCC 12055	8	64	32	64

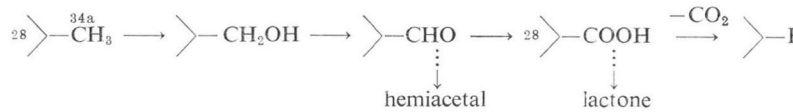
* W. WEHRLI, unpublished results.

Discussion

3-Hydroxyrifamycin S (**1**) and 3,31-dihydroxyrifamycin S (**2**) are the first active natural rifamycins to be isolated with a hydroxyl group in position 3 of the chromophore. Quite recently, 8-deoxy-3-hydroxyrifamycin S was isolated in our laboratories from the protorifamycin I producing mutant F 1/24 of *Nocardia mediterranei*, but owing to the missing hydroxyl group in position 8 this compound showed only marginal biological activity[8]. 3-Thiomethylrifamycin S[9] and rifamycins P, Q and Verde[10] are further examples of natural rifamycins with substituents in position 3 isolated during the last few years from ansamycin producing strains.

Modifications of the ansa chain through oxidation, hydroxylation or acetylation are very widespread among natural ansamycins. Numerous examples have been found among the compounds isolated from ansamycin-producing mutants. Starting from protorifamycin I for instance, a number of modifications in the ansa chain have been observed, such as oxidation of the C-23 hydroxyl group, hydroxylation at C-13, C-20 or C-30 and acetylation of the C-23 hydroxyl group[8]. 3,31-Dihydroxyrifamycin S, 30-hydroxyrifamycin W and 28-dehydroxymethyl-28,30-dihydroxyrifamycin W are now further examples of hydroxylations in the ansa chain. But while hydroxylation at C-28 or C-30 has been detected before in the streptovaricins[11], all of which have a hydroxyl group in position 28, rifamycin R (identical to 30-hydroxyrifamycin S)[12] and 30-hydroxyprotorifamycin I[8], hydroxylation at C-31 as in 3,31-dihydroxyrifamycin S (**2**) has not been reported so far. Further examples of ansamycins modified in the ansa chain are rifamycin Y[13] and streptovaricin J and F[11]. Since these above mentioned modifications can occur at almost every carbon atom of the ansa chain and with ansamycins of different chromophores, such as rifamycins S and W, protorifamycins and streptovaricins, one might assume that these hydroxylating enzymes do not act very specifically.

During the biosynthesis of rifamycin S, especially during the transformation of rifamycin W to rifamycin S, C-34a has to be eliminated. The existence of 28-formyl-rifamycin W and 28-carboxy-rifamycin W in form of their hemiacetal and lactone indicates the normal route of elimination of a methyl group by oxidation following decarboxylation:



This mechanism is supported by the formation of proansamycin B-M, with a C-34a methyl group, by the F 1/24 mutant[14], and by the isolation of rifamycin W (34a–CH_2OH)[5] and 28-dehydroxymethyl-28,30-dihydroxyrifamycin W (28–H).

The 3-hydroxy group can be introduced into 3-hydroxyrifamycin S (or 8-deoxy-3-hydroxyrifamycin S) by various pathways:

1. C-3-Hydroxylation of rifamycin S.
2. C-3-Hydroxylation of an early intermediate such as protorifamycin I[15] or proansamycin B[15].
3. Biosynthesis of the ansamycin from a starter unit already hydroxylated at the right position.

From the results of the transformation experiment described in the foregoing paper pathway 1 can be excluded[1]. Which of the two possible remaining pathways is operative can't be decided with the present knowledge.

16,17-Dehydrorifamycin G seems to be a metabolite of rifamycin S rather than a precursor, as was demonstrated earlier by LANCINI *et al.* with respect to rifamycin G[4].

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