EARLY INTERMEDIATES IN THE BIOSYNTHESIS OF ANSAMYCINS

III. ISOLATION AND IDENTIFICATION OF FURTHER 8-DEOXYANSAMYCINS OF THE RIFAMYCIN-TYPE

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A number of minor compounds were isolated from fermentations of the protorifamycin I producing strain *Nocardia mediterranei* F 1/24[1,2] and identified by means of chemical and spectroscopic methods. Two types of structures were identified:
Type 1: modified protorifamycins (derived from protorifamycin I)
Type 2: defective rifamycins (8-deoxyrifamycins).

As described earlier[1], protorifamycin I, the main product of *Nocardia mediterranei* F 1/24 is the earliest precursor for the ansamycins of the rifamycin-type isolated so far. Two minor compounds of the ansamycin-complex from mutant F 1/24 were identified as protorifamycin I-Ml[2] (degradation product of protorifamycin I) and proansamycin B-Ml[2] (degradation product of proansamycin B, the common progenitor for the naphthalenic ansamycins postulated in part I of this series of papers[1]). Protorifamycin I-Ml and proansamycin B-Ml are the first reported ansamycins with an opened ansa chain.*

In this final paper we describe nine further products of *Nocardia mediterranei* F 1/24 which we were able to identify (structures Fig. 1). Six of these minor compounds are direct modifications of protorifamycin I (type 1), namely: protorifamycin I-lactone, 23-ketoprotorifamycin I, 30-hydroxyprotorifamycin I, 20-hydroxyprotorifamycin I, 13-hydroxyprotorifamycin I and 23-acetoxyprotorifamycin I. Three of the identified minor compounds are defective rifamycins (type 2), namely: 8-deoxyrifamycin B, 8-deoxyrifamycin S (SV) and 8-deoxy-3-hydroxyrifamycin S (SV).

Experiments and Results

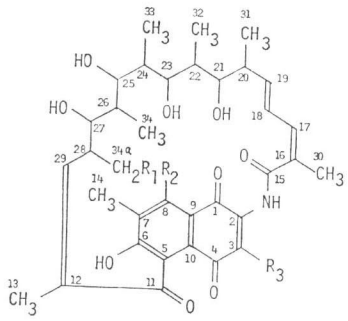
Fermentation, Isolation and Purification of the Minor Ansamycins

To obtain large enough amounts of the minor compounds from *Nocardia mediterranei* F 1/24 a fermentation was carried out in a 30-liter fermenter with liquid complex medium 151b as described in the preceding paper[2]. This fermentation yielded 78 g of crude ansamycin mixture after extraction.[2] By repeated chromatography on silica-gel columns using chloroform-methanol- and toluol-ethyl acetate-methanol-gradients the ansamycin-compounds were separated and enriched. Final purification was achieved by preparative TLC on silica-gel PF 254 plates (1.5 mm layer, Merck).

The following amounts of chromatographically pure ansamycins were obtained: protorifamycin I (16.9 g), proansamycin B-Ml[2] (190 mg), protorifamycin I-Ml[2] (60 mg), protorifamycin I-lactone (970 mg), 23-ketoprotorifamycin I (247 mg), 30-hydroxyprotorifamycin I (180 mg), 20-hydroxyprotorifamycin I (125 mg), 13-hydroxyprotorifamycin I (31 mg), 23-acetoxyprotorifamycin I (103 mg), 8-

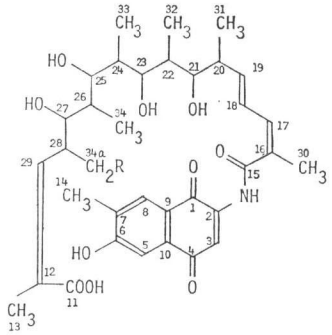
* Quite recently another open-chain analog in the streptovaricin series has also been reported[17].

Fig. 1. Structures of 8-deoxyansamycins and rifamycins.

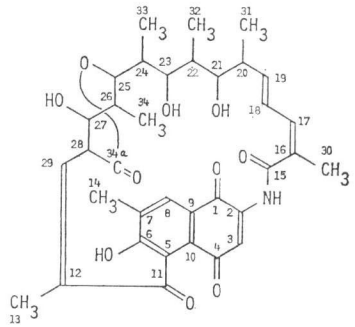


	R_1	R_2	R_3
"Proansamycin B"*	H	H	H
Protorifamycin I	OH	H	H
Protostreptovaricin I	H	H	CH_3
Rifamycin W	OH	OH	H

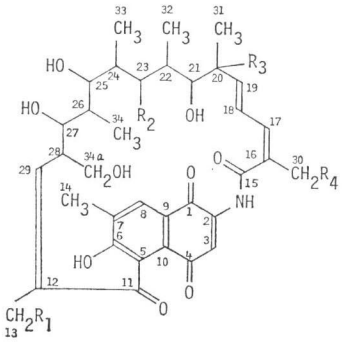
* hypothetical structure[1,2]



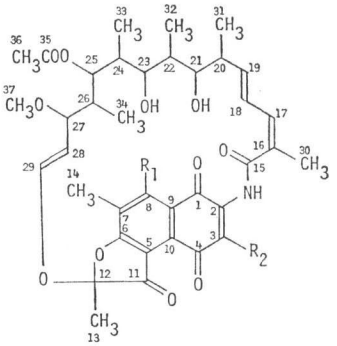
	R
Proansamycin B-M1	H
Protorifamycin I-M1	OH



Protorifamycin I-lactone



	R_1	R_2	R_3	R_4
23-Ketoprotorifamycin I	H	=O	H	H
30-Hydroxyprotorifamycin I	H	OH	H	OH
20-Hydroxyprotorifamycin I	H	OH	OH	H
13-Hydroxyprotorifamycin I	OH	OH	H	H
23-Acetoxyprotorifamycin I	H	OAc	H	H

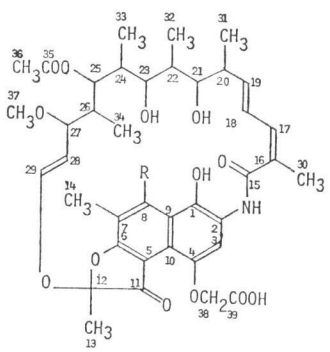


	R_1	R_2
8-Deoxyrifamycin S	H	H
8-Deoxy-3-hydroxyrifamycin S	H	OH
Rifamycin S	OH	H

deoxyrifamycin B (92 mg), 8-deoxyrifamycin S (282 mg) and 8-deoxy-3-hydroxyrifamycin S (280 mg).

Structures of the 8-Deoxyansamycins from *Nocardia mediterranei* F 1/24

All the compounds isolated from *Nocardia mediterranei* F 1/24 are 8-deoxyansamycins. Their structures have been elucidated by means of spectroscopical methods, especially by direct comparison of their ^{13}C-NMR and ^{1}H-NMR spectra with the corresponding spectra of protorifamycin I or rifamycin S respectively.



	R
8-Deoxyrifamycin B	H
Rifamycin B	OH

Table 1. ^{13}C-NMR data of the 8-deoxyansamycins from mutant F 1/24.
(δ values in ppm)

Carbon	Protorifamycin I (CD_3OD)[1)]	Protorifamycin I-lactone (CD_3OD)	23-Keto-protorifamycin I (CD_3OD)	20-Hydroxy-protorifamycin I (CD_3OD)	23-Acetoxy-protorifamycin I (CD_3OD)	8-Deoxy-3-hydroxy-rifamycin S ($CDCl_3$)	8-Deoxy-rifamycin S ($CDCl_3$)
C (1)	180.2	179.7	180.2	180.4	180.3	179.2	179.5
C (2)	140.8*	141.9	141.5*	141.1	140.9*	125.8	139.2
C (3)	118.2	115.6	118.6	118.3	118.3	146.1	116.0
C (4)	187.1	186.8	187.1	187.3	187.3	177.7	183.0
C (5)	131.3	131.3	132.1	131.6	131.4	116.8	116.4
C (6)	159.4	159.8	159.6	159.6	159.8	173.2	173.7
C (7)	133.0	133.9	133.2	133.2	133.1	129.0	129.8
C (8)	131.6	131.7	131.5	131.6	131.7	136.7	137.0
C (9)	124.0	124.0	123.6	123.9	124.0	118.9	125.9
C (10)	128.5	127.9	127.5	128.4	128.6	128.8	130.5
C (11)	200.2	198.7	200.0	200.4	200.3	193.5	193.6
C (12)	141.7*	144.1	141.8*	141.7	142.1*	108.1	108.4
C (15)	172.0	172.2	172.2	171.6	172.4	171.3	169.7
C (16)	131.9	133.2*	132.6	132.7	132.1	130.6	130.7
C (17)	135.1	135.2	133.3	135.3	134.8	135.2	133.6
C (18)	126.1	125.8	126.9	125.3	125.9	123.8	124.1
C (19)	141.5*	140.5	140.6	147.6	141.4*	143.0	142.1
C (29)	141.5*	133.4*	140.6	141.7	140.9*	144.4	144.5
C (21)	71.0	73.8	70.9	76.3*	74.5*	73.4	73.5
C (23)	74.7	78.9	220.8	71.3	76.0*	77.4	77.4
C (25)	68.8	80.9	67.9	68.9	67.7	73.1	73.5
C (27)	78.8	78.9	78.0	79.9	78.6	81.1	81.1
C (20)	39.0	39.5	42.2	75.9*	38.9	38.7	38.8
C (22)	34.1	33.8	} 48.7/49.5	34.3	34.2	32.9	32.8
C (24)	37.7	36.5		38.0	37.4	37.5	} 37.4/37.3
C (26)	43.8	40.4	43.0	43.6	42.3	37.5	
C (28)	49.3	53.0	49.5	49.3	48.9	116.4	117.0
C (13)	18.0	17.1	20.0	17.0	17.9	21.7	21.9
C (14)	17.0	17.1	16.9	17.0	17.0	15.1	15.0
C (30)	20.2	19.7	20.3	20.1	20.3	20.1	20.1
C (31)	11.1	11.8	11.6	25.9	11.2	17.0	16.9
C (32)	11.5	12.2	14.5	11.5	11.5	11.4	11.3
C (33)	8.8	9.4	8.2	9.0	9.5	8.9	8.8
C (34)	12.5	15.9	12.7	12.5	12.4	11.4	11.3
C (34a)	64.3	174.4	64.3	64.2	63.5	—	—
C (35)	—	—	—	—	174.6[a]	172.8[b]	173.1[b]
C (36)	—	—	—	—	20.9[a]	21.0[b]	21.0[b]
C (37)	—	—	—	—	—	56.9	56.9

* Tentative assignment

[a] Acetoxy group at C-23

[b] Acetoxy group at C-25

Further data: Rifamycin S see refs. 3 and 4. Proansamycin B-M1 and protorifamycin I-M1 see ref. 2.

The ^{13}C-NMR spectra of 13-hydroxyprotorifamycin I, 30-hydroxyprotorifamycin I and 8-deoxyrifamycin B were only of minor quality, but the most important signals could be assigned.

Table 2. 360-MHz NMR data of the 8-deoxyansamycins from mutant F 1/24. (δ values in ppm, coupling constants J (Hz) in brackets)

Protons	Protorifamycin I (CD_3OD)	Protorifamycin I-lactone (DMSO-d_6)	23-Keto-protorifamycin I (DMSO-d_6)	30-Hydroxyprotorifamycin I (CD_3OD)	20-Hydroxyprotorifamycin I (CD_3OD)	13-Hydroxyprotorifamycin I (CD_3OD)	23-Acetoxyprotorifamycin I (CD_3OD)	8-Deoxyrifamycin B (CD_3OD)	8-Deoxyrifamycin S (CD_3Cl)	8-Deoxy-3-hydroxyrifamycin S (CD_3Cl)
H (3)	7.52s	7.3s	7.49s	7.60s	7.60s	7.60s	7.60s	7.8s	7.3s	—
H (8)	7.9s	7.8s	7.88s	7.95s	7.95s	7.97s	7.95s	8.45s	8.18s	8.16s
H (17)	6.15d (9)	6.33d (16)	6.21d (12)	6.5d (9)	5.9d (16)	6.24d (12) DR	6.2d (16)	6.2d*	6.1d (12)	6.1d (12)
H (18)	6.25dd (9/16)	6.33dd (12/16)	5.86dd (12/16)	6.85dd (9/16)	6.40dd (9/16)	6.47dd (12/16)	6.40dd (12/16) DR	6.2dd*	6.3dd (12/16)	6.3dd (12/16)
H (19)	5.97dd (6/12)	5.72dd (12/16) DR	5.74dd (12/16) DR	6.35dd (9/16)	6.23d (9)	6.05dd (9/16)	6.05dd (6/16) DR	5.87dd (6/16)	6.0dd (6/16)	6.0dd (6/16)
H (21)	3.75dd (6)	3.55d (9) DR	3.40dd* (6/8) DR	n.l.	3.9d (9)	3.95dd (2/9)	3.95d (12) DR	3.6d (9)	3.8dd (1/9)	3.95dd (1/9)
H (23)	~3.3dd*	3.65dd (3/9) DR	—	n.l.	~3.3*	~3.3*	5.25d (12)	3.1*	3.3*	~3.35dd
H (25)	3.57dd	4.00d (9) DR	3.67dd (3/9)	n.l.	n.l.	n.l.	n.l.	4.25d (9)	4.8d (10)	~4.8d*
H (27)	~3.3*	3.17dd (6/9) DR	4.24d (6)	n.l.	~3.3*	~3.3*	n.l.	3.2dd (2/9)	3.3dd (2/8)	~3.1*
H (28)	n.l.	3.5d (6) DR	~2.4m*	n.l.	n.l.	n.l.	2.50m	5.1dd (9/12)	5.15dd (8/12)	5.08dd (4/12)
H (29)	6.15d (9)	6.12d (6) DR	6.03d (9)	6.3d (9) DR	6.27d (9)	6.54d (12) DR	5.28d (9)	6.2d*	6.2d (12)	6.15d (12)

H (34a)	3.2AB*	—	n.l.*	2.85AB	2.80AB	2.85AB	n.l.*	—	—	—
CH_3(13)	2.0s	1.93s	1.94s	2.1s DR	2.07s	4.56AB DR	2.1s	1.75s	1.70s	1.72s
CH_3(14)	2.35s	2.33s	2.32s	2.40s	2.33s	2.38s	2.36s	2.40s	2.50s	2.5s
CH_3(30)	1.95s	2.02s	1.90s	4.30AB	2.07s	2.08s DR	2.1s	2.04s	2.02s	2.04s
CH_3(31)	0.84d (6)	0.77d (6) DR	1.00d (7) DR	0.95d (6)	1.2s	0.9d (7)	0.86d (7)	0.85d (6)	0.80d (7)	0.82d (7)
CH_3(32)	0.95d (6)	0.87d (6)	0.93d (7) DR	1.07d (6)	1.15d (6)	1.05d (7)	1.03d (7)	1.07d (6)	1.00d (7)	1.0d (7)
CH_3(33)	0.58d (6)	0.77d (6)	1.07d (7)	0.75d (6)	0.73d (6)	0.71d (7)	0.81d (7)	0.57d (6)	0.6d (7)	0.62d (7)
CH_3(34)	0.2d (6)	0.67d (6)	0.19d (7)	0.42d (6)	0.40d (6)	0.40d (7)	0.47d (7)	−0.2d (6)	~0.1d*	~0.1d*
H (20)		2.15m DR	1.69dd DR				2.35m DR			
H (22)		1.62m	2.65dd DR							
H (24)			2.4m*							
H (26)										
OCH_3(37)								3.1s	3.02s	3.01s
CH_3CO								2.02s	1.96s	1.98s
$-OCH_2COOH$								4.75AB		

DR: position confirmed by double resonance experiment
n.l.: signal present but not located
* Signal is overlapped by other signals.

By this comparison most of the carbons in the ^{13}C-NMR spectra as well as most of the protons in the ^{1}H-NMR spectra could be assigned (see Tables 1 and 2). In the ^{1}H-NMR spectra of the 8-deoxyrifamycins of the protorifamycin I-type (type 1) the aromatic proton H-8 was normally found at 7.9 ppm. The structures of the new 8-deoxyansamycins are discussed in detail below.

Protorifamycin I-lactone ($C_{35}H_{41}NO_{10}$, M^+ at *m/e* 635)

In protorifamycin I-lactone the C-34a hydroxymethyl group of protorifamycin I at C-28 is oxidized to a carboxylic group which forms a lactone with the C-25 hydroxyl group. The ^{13}C-NMR spectrum is in good agreement with the postulated structure. The signal of C-34a found at 64.3 ppm in protorifamycin I has shifted downfield to 174.4 ppm corresponding there to a carbonyl signal. In addition the signals of C-25 and C-28 shifted to lower field, too, whereas for C-29 a small upfield shift was observed. In the 360 MHz-NMR spectrum of the lactone the proton H-28 was found at 3.5 ppm (doublet), whereas the AB-system of the H-34a protons is missing. The coupling of H-28 with the olefinic proton H-29 at 6.12 ppm was demonstrated by a decoupling experiment. In addition the exact positions of the protons H-20 to H-27 were confirmed by double resonance experiments.

23-Ketoprotorifamycin I ($C_{35}H_{43}NO_{10}$, M^+ of the persilylated product at *m/e* 1071 corresponding to a molecular weight of 637).

The structure assignment of 23-ketoprotorifamycin I is based on the following observations:

In the ^{13}C-NMR spectrum only four signals of C-atoms beside oxygen are present: C-21, C-25, C-27 and C-34a.

The signal of C-23 found at 74 ppm in protorifamycin I has shifted downfield to 220.8 ppm corresponding there to a ketogroup.

In the 360 MHz-NMR spectrum the signal of the H-23 proton is missing, whereas the proton H-21 was localized as a double doublet at 3.40 ppm. Therefore a ketogroup at C-21 can be excluded.

In addition the coupling of the H-21 proton with H-20 (1.69 ppm) and H-22 (2.65 ppm) as well as the coupling of H-20 with H-19 (5.74 ppm) and the C-31 methyl group (1.00 ppm) was demonstrated by decoupling experiments.

30-Hydroxyprotorifamycin I ($C_{35}H_{41}NO_{11}$, FD-MS: M^+ at *m/e* 655)

The structure differs from protorifamycin I only by its primary hydroxyl group at C-30 as evidenced by lacking the signal of the C-30 vinylic methyl group found at 20.2 ppm in the ^{13}C-NMR spectrum of protorifamycin I. In the 360 MHz-NMR spectrum only one vinylic methyl group is present (2.1 ppm). Its long range coupling with the olefinic proton H-29 could be demonstrated by a decoupling experiment. Therefore the remaining vinylic methyl group has to be the C-13 one. On the other hand a new AB-system at 4.30 ppm can be attributed to the C-30 hydroxymethyl group.

20-Hydroxyprotorifamycin I ($C_{35}H_{41}NO_{11}$, FD-MS: M^+ at *m/e* 655)

The structure assignment of 20-hydroxyprotorifamycin I is based on the fact that in the ^{13}C-NMR spectrum the C-20 signal found at 39 ppm in protorifamycin I is missing, whereas C-22, C-24, C-26 and C-28 show their normal chemical shifts. On the other hand due to the hydroxyl group at C-20 an additional signal was localized at 75.9 ppm. In addition small downfield shifts were observed for the C-31 methyl group as well as for C-19. In the 360 MHz-NMR spectrum only three doublets of secondary methyl groups are visible instead of four (C-32, C-33 and C-34). The C-31 methyl group forms now a singlet at 1.2 ppm. Finally the hydroxyl group at C-20 is responsible for a small downfield shift of the olefinic H-19 proton to 6.23 ppm where it was localized as a doublet instead of a double doublet as in the

former rifamycins. The same effect is observed for H-21, too.

13-Hydroxyprotorifamycin I ($C_{35}H_{41}NO_{11}$, FD-MS: M^+ at *m/e* 655)

13-Hydroxyprotorifamycin I is the second example where a vinylic methyl group in the ansa chain is oxidized to a primary alcohol. The structure assignment is based mainly on the 360 MHz-NMR spectrum where one of the signals of the vinylic methyl groups is missing. The long range coupling of the remaining vinylic methyl group at 2.08 ppm with the olefinic H-17 proton at 6.24 ppm was demonstrated by a double resonance experiment, thus proving that the missing signal of the vinylic methyl group has to be attributed to C-13. In addition the signal of the C-13 hydroxymethyl group was found as an AB-system at 4.56 ppm. Its long range coupling with the olefinic H-29 proton at 6.54 ppm was shown by an irradiation experiment.

23-Acetoxyprotorifamycin I ($C_{37}H_{47}NO_{11}$, FD-MS: M^+ at *m/e* 681)

The tentative structure assignment is based on the following observations:

In the ^{13}C-NMR as well as in the ^{1}H-NMR spectra an additional acetate group was observed.

Decoupling experiments showed the coupling between the olefinic H-19 proton at 6.05 ppm and the H-20 proton at 2.35 ppm on one hand and between the H-20 proton and the H-21 proton at 3.95 ppm on the other hand, thus proving that the hydroxyl group at C-21 is not acetylated.

The doublet at 5.25 ppm in the ^{1}H-NMR spectrum is attributed to the H-23 proton indicating an acetoxy group at C-23.

8-Deoxyrifamycin B ($C_{39}H_{49}NO_{13}$, MS: M^+ at *m/e* 721)

8-Deoxyrifamycin B was identified by its almost identical 360 MHz-NMR spectrum compared to rifamycin B except for the presence of the aromatic H-8 proton at 8.45 ppm. The methylene group of the glycolic acid was found as a singlet at 4.75 ppm.

8-Deoxyrifamycin S ($C_{37}H_{45}NO_{11}$, MS: M^+ at *m/e* 679)

This product was isolated in the oxidized form as a quinone (8-deoxyrifamycin S) and in the reduced form as a hydroquinone (8-deoxyrifamycin SV). The spectra were carried out with the oxidized form. The ^{13}C-NMR spectrum shows the same chemical shifts for the C-atoms of the ansa chain as in rifamycin S.[3,4] In the 360 MHz-NMR spectrum the aromatic H-8 proton is still present at 8.18 ppm and the H-3 proton was found at 7.3 ppm. No serious shift differences were observed for the rest of the protons compared to rifamycin S.[5]

8-Deoxy-3-hydroxyrifamycin S ($C_{37}H_{45}NO_{12}$, FD-MS of the persilylated product: M^+ at *m/e* 911 with 3 silylated groups corresponding to a molecular weight of 695).

Spectra were carried out with the oxidized form. In the 360 MHz-NMR spectrum the signal of the H-3 proton has disappeared, whereas the H-8 proton is still present at 8.16 ppm. The rest of the ^{1}H-NMR spectrum is almost identical with that of rifamycin S. From the molecular weight which is 16 mass units higher than for 8-deoxyrifamycin S it was concluded that C-3 is substituted by an additional hydroxyl group. The postulated structure was supported by the ^{13}C-NMR spectrum. Compared to 8-deoxyrifamycin S or rifamycin S strong shift differences were observed for the aromatic carbons C-2, C-3 and C-4 due to the hydroxyl group in position 3. With these findings the structure of 8-deoxy-3-hydroxyrifamycin S seems to be well established.

Biological Activity

The 8-deoxyansamycins isolated from *Nocardia mediterranei* F 1/24 showed no or very weak activity against Gram-positive bacteria, Gram-negative bacteria and *Candida albicans*.

For the products of type 1 this is not surprising because rifamycin W was found to be inactive, too. In the case of rifamycin W it was assumed that the lack of activity is due to an unsuitable spatial relationship between the hydroxyls at C-8, C-21 and C-23.[6] By investigating chemical derivatives of rifamycin S (SV), tolypomycin Y, streptovaricin C and rifampicin it was found that the oxygens at C-1 and C-8 (as hydroxyls or as quinoid oxygen) and the two hydroxyls at C-21 and C-23 must be involved in the formation of the complex between the active ansamycin and the bacterial RNA-polymerase[7~9]. Derivatization at C-8 (*e.g.* acetylation) yields inactive products[7~9]. The isolation of the inactive 8-deoxyansamycins of type 2, especially of 8-deoxyrifamycin S and 8-deoxy-3-hydroxyrifamycin S is a direct proof that the oxygen function at C-8 is essential for the biological activity of the naphthalenic ansamycins and does fully confirm the data from analyzing derivatives.

Discussion

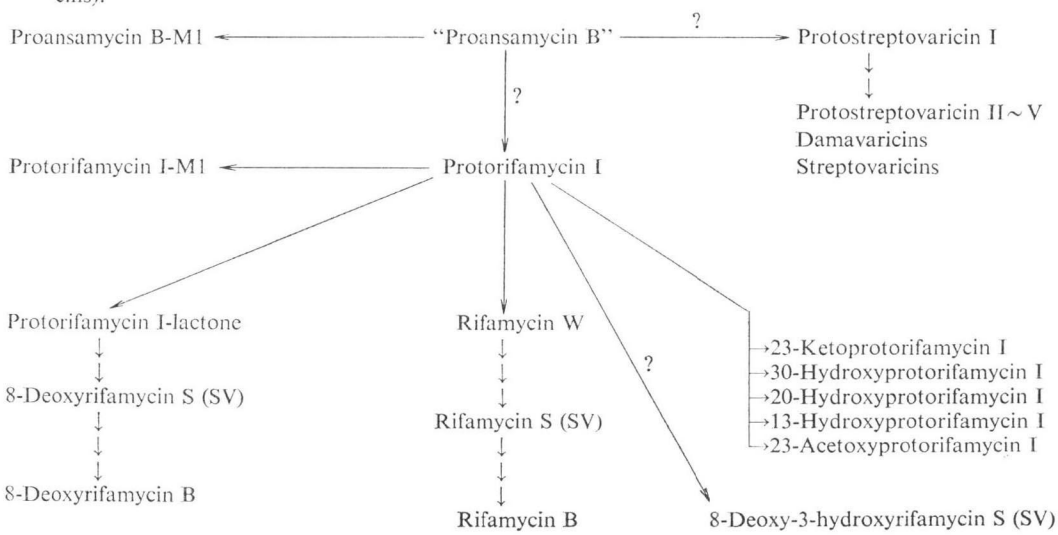
The structures of the compounds isolated from *Nocardia mediterranei* F 1/24 supply us with considerable biosynthetic information.

1. Protorifamycin I, the earliest direct precursor for the ansamycins of the rifamycin-type, is structurally related to protostreptovaricin I[10]. This observation led to the postulation of proansamycin B as a possible common precursor for the naphthalenic ansamycins.[1]

2. Our ability to isolate proansamycin B-M1 (degradation product of proansamycin B) is an indirect argument for the existence of proansamycin B and for the biosynthetic hypothesis of a common progenitor. The existence of protorifamycin I-M1 (degradation product of protorifamycin I) indicates that the cleavage of the bond between C-5 and C-11 is a non-specific (enzymatic ?) reaction for ansamycins with a protorifamycin I-type chromophore.[2]

3. Starting from protorifamycin I a number of modifications in the ansa chain (products of type 1, see Fig. 2) 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. Similar modifications are known for some end-products of the rifamycin group. Hydroxylation at C-20 or C-30 has been found before in rifamycin Y[11] or rifamycin R[12], respectively, whereas the hydroxylation at C-13 and the acetylation of the C-23 hydroxyl group has never been detected so far. However, most of the active rifamycins *e.g.* S, SV and B bear an acetoxy group at C-25. All these rifamycin-data together with the information from the

Fig. 2. The biogenetic relationships among the known 8-deoxyansamycins and the rifamycins (streptovaricins).



other ansamycin groups indicate that hydroxylation is possible at almost every methyl group of the ansa chain and even hydroxylation at trisubstituted carbons of the ansa chain can occur. It seems that the hydroxylating enzymes are not very specific, because the hydroxylations occur at many different positions and with ansamycins having very different chromophores, such as protorifamycin I or rifamycin S or streptovaricins.

4. The existence of 28-carboxyprotorifamycin I in form of the isolated protorifamycin I-lactone indicates that the elimination of C-34a during the transformation: protorifamycin I→8-deoxyrifamycin S (or rifamycin W→rifamycin S) follows the normal route for elimination of methyl by decarboxylation:

$$28\rangle\text{-}\overset{34a}{C}H_3 \rightarrow \rangle\text{-}CH_2OH \rightarrow \rangle\text{-}CHO \rightarrow \rangle\text{-}COOH \rightarrow \rangle\text{-}H$$

5. The formation of protorifamycin I-lactone together with 8-deoxyrifamycin S, 8-deoxy-3-hydroxyrifamycin S and 8-deoxyrifamycin B proves that the enzyme systems for the elimination of C-34a, for the ring extention (introduction of oxygen between C-12 and C-29), for the formation of the five-membered ring in the chromophore and for the transformation: 8-deoxyrifamycin S→8-deoxyrifamycin B (or rifamycin S→rifamycin B) accept both the 8-deoxy- and the 8-hydroxy-products as substrates. Protorifamycin I, however, is not as good as substrate as rifamycin W and the transformation protorifamycin I→8-deoxyrifamycin B is very slow. In *Nocardia mediterranei* F 1/24 only a small part (approximately 10%) of the synthesized protorifamycin I is transformed into protorifamycin I-lactone, 8-deoxyrifamycin S, 8-deoxy-3-hydroxyrifamycin S and 8-deoxyrifamycin B during the fermentation; the rest is accumulated in the culture broth (or modified by hydroxylation *etc.* to approx. 5%), in contrast to the original strain N813 (rifamycin B producer) where the transformation: rifamycin W→rifamycin B is almost complete and no significant accumulation of precursors such as the corresponding rifamycin W[13] can be observed.

In the streptovaricin-producing organism *Streptomyces spectabilis* all these enzyme systems mentioned above seem to be absent (or non-functional) because all the known ansamycins of the streptovaricin-type bear an unmodified C-28 methyl group C-34a, have an uncleaved carbon ansa chain with no oxygen between C-12 and C-29, no five-membered ring in the chromophore and no glycolic acid at C-4[10,14].

6. In the case of 8-deoxy-3-hydroxyrifamycin S different biogenetic origins can be postulated:

C-3 hydroxylation of 8-deoxyrifamycin S.

C-3 hydroxylation of a precursor such as protorifamycin I or proansamycin B followed by transformation into 8-deoxy-3-hydroxyrifamycin S.

Ansamycin biosynthesis beginning with a seven-carbon amino starter unit different from that for the normal rifamycins and streptovaricins (starter unit hydroxylated in the position which later becomes position 3 of the final ansamycins)[15,16].

With the present knowledge we can not decide which of these possible pathways is correct.

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