# EARLY INTERMEDIATES IN THE BIOSYNTHESIS OF ANSAMYCINS
## I. ISOLATION AND IDENTIFICATION OF PROTORIFAMYCIN I

ORESTE GHISALBA, PETER TRAXLER and JAKOB NÜESCH

Research Laboratories of the Pharmaceuticals Division of CIBA-GEIGY Limited, Basel, Switzerland

(Received for publication June 5, 1978)

A mutant strain derived from *Nocardia mediterranei* N813 was found to accumulate a number of novel ansamycins structurally related to the protostreptovaricins and to rifamycin W. One of the main components of this ansamycin complex, protorifamycin I (8-deoxyrifamycin W), was purified and identified by means of chemical and spectroscopic methods.

For the biosynthesis of the ansamycins containing a naphthalenic moiety (rifamycins, streptovaricins, tolypomycins, halomicins) a common progenitor was proposed by WHITE *et al.*[1,2]. This hypothesis was based on the identical incorporation patterns of $^{13}C$ enriched precursors found for rifamycin W[2], rifamycin S[1] and streptovaricin D.[3] So far there are no biosynthetic data on naphthalenic ansamycins which contradict this proposal, but during the last few years a number of early intermediates in ansamycin biogenesis have been isolated from mutants of the original ansamycin-producing strains. The structures of these compounds strongly support the idea of a common progenitor.

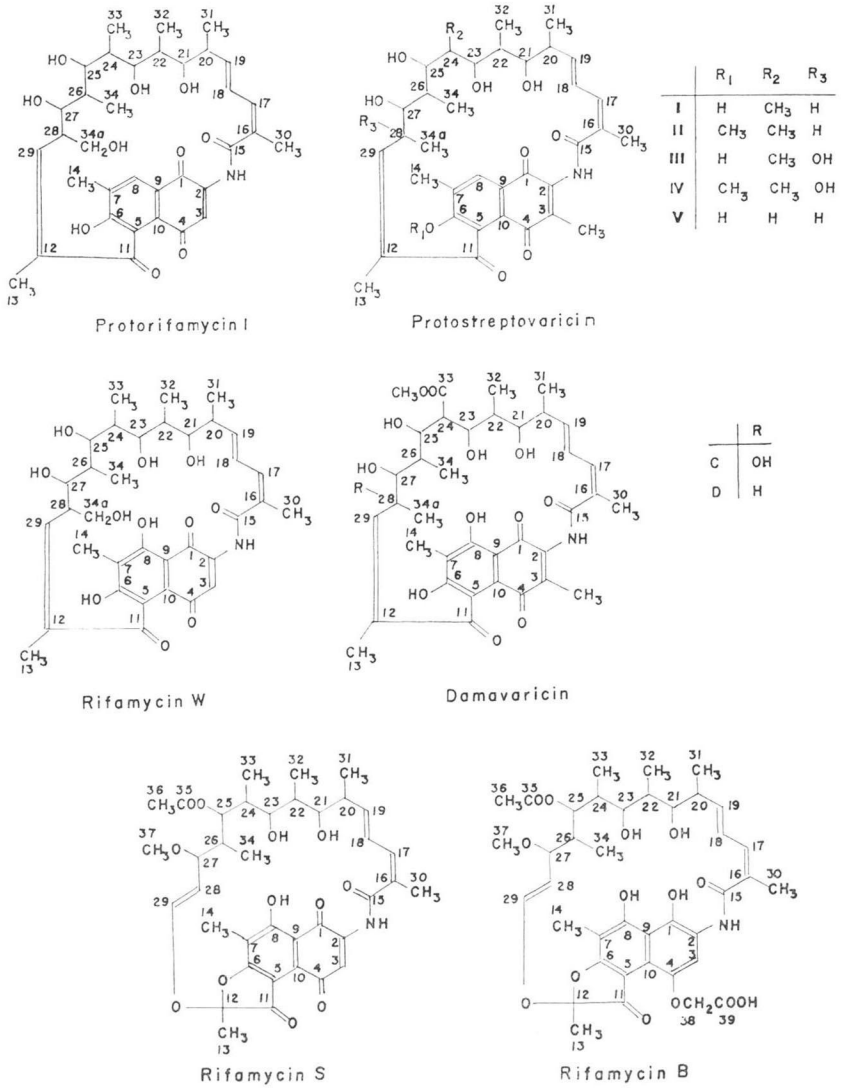
From a mutant strain of *Streptomyces spectabilis* RINEHART *et al.*[4] isolated damavaricins C and D as biologically active apparent precursors of the streptovaricins. DESHMUKH *et al.*[5] isolated protostreptovaricins I~V as minor compounds of the streptovaricin complex. Protostreptovaricins are considered to be precursors for both damavaricins and streptovaricins. Protostreptovaricin I is the earliest precursor for the ansamycins of the streptovaricin group known so far. The protostreptovaricins contain the complete carbon skeleton of the streptovaricins but lack much of their oxygenation. The most striking difference of protostreptovaricins from streptovaricins is their lack of the hydroxyl group at C-8 of the chromophore (numbering analogous to the rifamycins).

Also, in naphthomycin[6] (revised structure[5,7]) the hydroxyl group at C-8 is lacking. However, naphthomycin with its larger ansa chain cannot be derived from the common progenitor mentioned above. Rifamycin W, isolated by WHITE *et al.*[2,8], was shown to be a precursor of rifamycin S and all the other rifamycins.

The biologically inactive protorifamycin I described in this paper is a direct biogenetic precursor of rifamycin W. Its structure is almost identical with that of rifamycin W except that the hydroxyl group at C-8 of the chromophore is lacking. Protorifamycin I is therefore not only related to rifamycin W but also to the protostreptovaricins I and III (protostreptovaricin I lacks the hydroxyl group at C-34a and has an additional methyl group at C-3 originating from methionine; protostreptovaricin III has a hydroxyl group at C-28 instead of C-34a and an additional methyl group at C-3).

Protorifamycin I and protostreptovaricin I thus differ only in the substitutions at C-3 and at C-34a and should therefore be closely related to the hypothetical common progenitor of the naphthalenic ansamycins.

Fig. 1. Structures of protorifamycin I, rifamycins W, S and B, protostreptovaricins I~V and damavaricins C and D.




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| I | H | $CH_3$ | H |
| II | $CH_3$ | $CH_3$ | H |
| III | H | $CH_3$ | OH |
| IV | $CH_3$ | $CH_3$ | OH |
| V | H | H | H |

| | R |
|---|---|
| C | OH |
| D | H |

## Experiments and Results

### Mutant Selection and Preliminary Investigations

The protorifamycin I producing strain *Nocardia mediterranei* F 1/24 is a mutant of *Nocardia mediterranei* N813 (a high rifamycin B producer from the laboratories of Ciba-Geigy Ltd.). Strain F 1/24 was obtained by UV-irradiation using the same techniques as for the induction of auxotrophic mutants of *Nocardia mediterranei* described by SCHUPP[9] and GHISALBA[10] followed by a screening for colour variations of the colonies. Colonies of strain F 1/24 on agar plates show a dark red-brown colour clearly different from the orange colonies of strain N813.

One colony of strain F 1/24 was transferred into a 200-ml shake flask with 40 ml of liquid complex medium 148 (22 g glucose, 5 g Lab-Lemco beef extract (Oxoid), 5 g peptone C, 5 g yeast extract, 3 g

Bacto-Casitone (Difco) and 1.5 g NaCl in 1 liter of water; pH 6.5 after sterilization for 20 minutes at 120°C) and fermented for 7 days on a shaker (250 rpm, 28°C). Then the mycelium was harvested, washed with 0.066 M phosphate buffer, pH 7, resuspended with 20 ml of skim milk, frozen in portions of 3 ml (60 mg dry weight) and stored at −30°C.

The dark red culture filtrate (pH 7.5~8.0) was acidified (pH 2 with 2 N sulfuric acid) and extracted with an equal amount of ethyl acetate and the ethyl acetate extract was concentrated to 1/10 of the original volume under reduced pressure at 50°C. The concentrate was investigated by TLC on precoated silica gel 60 F-254 plates (Merck) using 1-butanol - acetic acid - water (80: 20: 20) and chloroform - methanol (95: 5) as solvent systems. No rifamycin B or S, and no rifamycin W, but a number of other red and yellow compounds could be detected.

The concentrate was then evaporated to dryness and equal parts of the residue were dissolved in water, ethyl acetate, phosphate buffer (pH 8) and MICHAELIS buffer (pH 4.65). The UV-vis spectra were recorded. No maxima between 420 and 700 nm were detectable with water or MICHAELIS buffer pH 4.65 (yellow solutions). With ethyl acetate or phosphate buffer pH 8 (red-blue solutions) maxima at 500~510 nm, 530~540 nm and 565~575 nm occurred. This pH-sensitivity is typical for rifamycins with an oxidized naphthoquinoid chromophore. Rifamycin S and rifamycin W in phosphate buffer pH 8 show maxima at 520 nm or 540 nm respectively. Rifamycins with a reduced naphthoquinoid chromophore such as rifamycin B or rifamycin SV do not show this pH-sensitivity and have no maxima above 450 nm, not even in 0.1 N NaOH.

Therefore we could assume that *Nocardia mediterranei* F 1/24 accumulates a number of ansamycins with an oxidized naphthoquinoid chromophore. However, in contrast to rifamycin S and rifamycin W the mixture of the products of strain F 1/24 not only shows the maxima above 450 nm in phosphate buffer pH 8 but also in ethyl acetate. Some of these products must thus be different from all the other rifamycins known so far.

## Fermentation, Isolation and Purification of Protorifamycin I

A 500-ml shake flask with 100 ml of liquid complex medium 148 was inoculated with mycelium of *Nocardia mediterranei* F 1/24 (120 mg total dry weight) and fermented for 4 days (250 rpm, 28°C). Five ml of this culture were transferred into each of 20 500-ml shake flasks (one baffle) with 100 ml of industrial fermentation medium 151b (70 g glucose, 20 g glycerol, 30 g protanimal, 10 g soybean meal, 8 g $CaCO_3$, 3 g $(NH_4)_2SO_4$, 1 g $KH_2PO_4$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.003 g $CuSO_4 \cdot 5H_2O$, 0.05 g $ZnSO_4 \cdot 7H_2O$, 0.004 g $MnSO_4 \cdot 4H_2O$, 0.002 g $CoCl_2 \cdot 6H_2O$ and 0.001 g $(NH_4)_2Mo_7O_{24} \cdot 4H_2O$ in 1 liter of water; pH 7.1~7.3 after sterilization for 20 minutes at 120°C) and fermented for another 9 days (250 rpm, 28°C). The mycelium was separated by centrifugation (20 minutes, 3,000 $\times g$) and washed with water. The supernatants were collected, acidified (pH 2 with sulfuric acid) and extracted three times with equal amounts of ethyl acetate. The three extracts were combined and evaporated to dryness under reduced pressure at 50°C and 8.5 g of reddish brown substance was obtained.

A first fractionation of this crude mixture was achieved by chromatography on silica gel (600-g column) with chloroform and increasing amounts of acetone and afterwards methanol as eluents. The eluate fractions were assayed by TLC on Antec SL 254 silica gel 60 plates (Merck) using chloroform - methanol (80: 20) as solvent system. Protorifamycin I (Rf 0.30~0.31) was found as the main component together with three minor compounds in the fractions with chloroform - acetone (20: 80),

pure acetone - methanol (95: 5) as eluents. These fractions were pooled and evaporated to dryness; 1.2 g of crude protorifamycin I was obtained.

Final purification was achieved by a second chromatography on silica gel (120-g column) with chloroform and increasing amounts of methanol as eluents. Protorifamycin I was found in the fractions with chloroform - methanol (90: 10) as eluent. From these fractions 450 mg of chromatographically pure protorifamycin I and 330 mg of protorifamycin I with minor impurities were obtained.

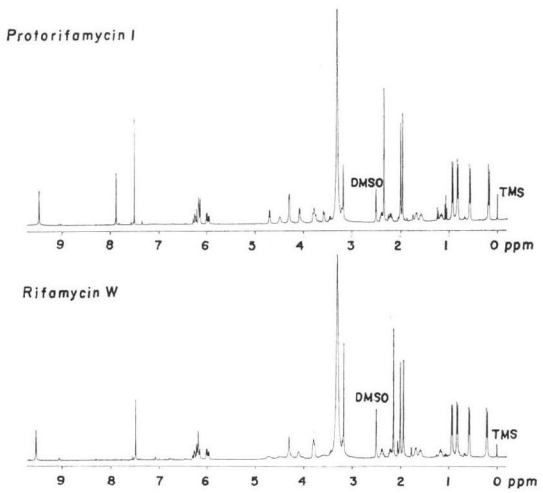
## Physico-Chemical Properties and Structure of Protorifamycin I

Protorifamycin I forms yellow crystals from ethyl acetate (melting point 175~180°C, $[\alpha]_D^{20}$ ($CH_3OH$) $+227\pm1°$). By potentiometric titration in 80% ethylene glycol monomethyl ether with 0.1 N trimethylammonium-hydroxide two ionizable functions were found (one is very weak). The first of these two functions has a pKa of 7.13. The UV spectrum of protorifamycin I shows variation of the maxima depending on the pH; spectrum in ethanol pH 8: 221 (ε 33,000), 315 (21,800), 558 (2,900) nm; in ethanol pH 4: 227 (ε 31,300), 273 (25,000), 308 (13,500) nm, shoulder at 344 nm; in ethyl acetate: 274 (ε 24,500), 305 (17,200) nm, shoulder at 348 nm; in 0.1 N NaOH: 299 (ε 17,500), 390 (8,800), 548 (960) and 600 (580) nm. The IR spectrum of protorifamycin I lacks the bands at 1725 $cm^{-1}$ and 1700 $cm^{-1}$ which are due to the carbonyls of the dihydrofuranone ring and of the acetoxy group in rifamycin S. A band at 1660 $cm^{-1}$ can be attributed to the quinone carbonyl at C-4, whereas the band of the quinone carbonyl at C-1 linked in an intramolecular hydrogen bond, which appears at 1630 $cm^{-1}$ in rifamycin W, is missing in the IR spectrum of protorifamycin I. This already indicates some structural difference in the aromatic part of the molecule.

Micro-hydrogenation of protorifamycin I over platinum oxide in acetic acid gave an uptake of 2.8 moles of hydrogen.

From elemental analyses and from the data of $^{13}$C-NMR and mass spectroscopy the molecular formula of protorifamycin I was deduced to be $C_{35}H_{45}NO_{10}$. Elemental analyses: calculated for $C_{35}H_{45}NO_{10}$: C 65.71%, H 7.09%, N 2.19%, O 250.1% and found for protorifamycin I: C 63.55%, H 7.25%, N 2.55%, S none. In the FD mass spectrum of protorifamycin I a molecular ion at *m/e* 639 is visible which was found at *m/e* 1143 in the mass spectrum of the persilylated product. This indicates the presence of seven exchangeable protons corresponding to six hydroxyl groups and one amide group. The chromophoric ions of protorifamycin I at *m/e* 230 and 258 have 16 mass units less than the chromophoric ions of rifamycin W[11,12], which is another good argument for structural differences in the aromatic part of the molecule. In the $^{13}$C-NMR spectrum the signals of 35 carbon atoms were found.

Fig. 2. 360 MHz NMR spectra of protorifamycin I and rifamycin W (DMSO-$d_6$).



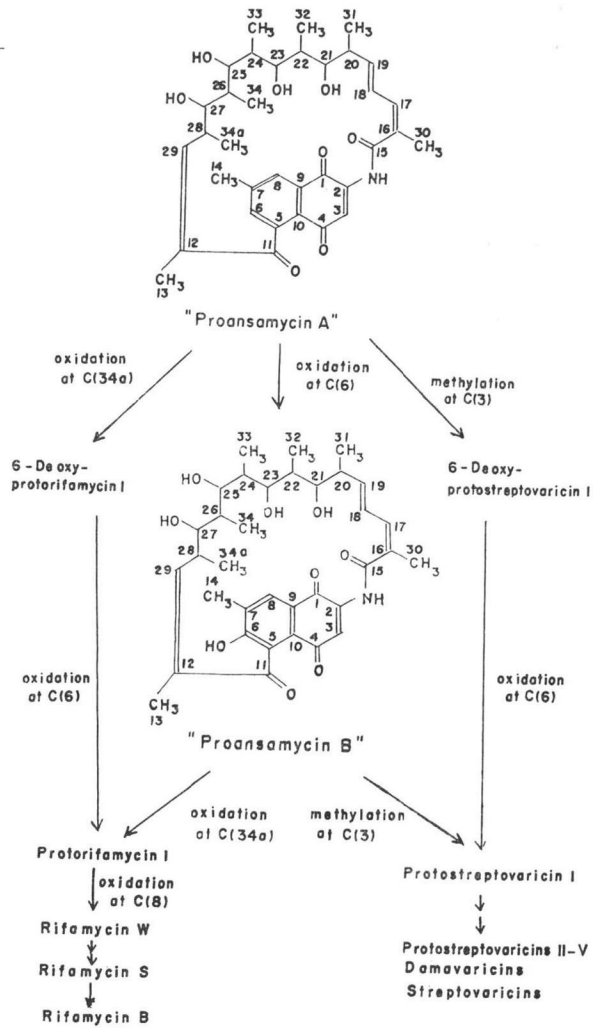

The postulated structure of protorifamycin I (see Fig. 1) was finally confirmed by comparison of the $^1$H-NMR and $^{13}$C-NMR spectra of

Table 1. $^{13}$C-NMR data of rifamycin W, protorifamycin I and 6-O-methylprotorifamycin I (all spectra in $CD_3OD$).

| Carbon | Rifamycin W ppm | Protorifamycin I ppm | 6-O-Methylprotorifamycin I ppm |
|---|---|---|---|
| C (1) | 183.8 | 180.2 | 180.5 |
| C (2)* | 140.5 | 140.8 | 140.3 |
| C (3) | 118.4 | 118.2 | 118.7 |
| C (4) | 186.3 | 187.1 | 186.4 |
| C (5) | 124.8 | 131.3 | 135.5 |
| C (6) | 161.2 | 159.4 | 162.0 |
| C (7) | 119.2 | 133.0 | 139.6 |
| C (8) | 163.6 | 131.6 | 132.0 |
| C (9) | 108.1 | 124.0 | 127.8 |
| C (10) | 129.6 | 128.5 | 131.0 |
| C (11) | 199.8 | 200.2 | 198.9 |
| C (12)* | 140.7 | 141.7 | 141.8 |
| C (13) | 18.0 | 18.0 | 18.0 |
| C (14) | 8.6 | 17.0 | 16.9 |
| C (15) | 171.8 | 172.0 | 172.1 |
| C (16) | 131.8 | 131.9 | 132.0 |
| C (17) | 135.2 | 135.1 | 134.9 |
| C (18) | 126.0 | 126.1 | 126.0 |
| C (19)* | 142.0 | 141.5 | 142.6 |
| C (20) | 38.9 | 39.0 | 39.0 |
| C (21) | 71.0 | 71.0 | 71.0 |
| C (22) | 34.1 | 34.1 | 34.2 |
| C (23) | 74.7 | 74.7 | 74.6 |
| C (24) | 37.7 | 37.7 | 37.7 |
| C (25) | 68.8 | 68.8 | 68.5 |
| C (26) | 43.8 | 43.8 | 43.8 |
| C (27) | 78.8 | 78.8 | 78.9 |
| C (28) | 49.2 | 49.3 | 49.2 |
| C (29)* | 141.5 | 141.5 | 141.4 |
| C (30) | 20.2 | 20.2 | 20.2 |
| C (31)* | 11.1 | 11.1 | 11.1 |
| C (32)* | 11.5 | 11.5 | 11.5 |
| C (33) | 8.8 | 8.8 | 8.9 |
| C (34)* | 12.6 | 12.5 | 12.4 |
| C (34a) | 64.2 | 64.3 | 64.1 |
| C (35) 6-O-$CH_3$ | — | — | 62.8 |

* tentative assignments

Fig. 3. Possible pathways for the biosynthesis of protorifamycin I and protostreptovaricin I starting from the hypothetical common progenitors "proansamycin A" and "proansamycin B".




protorifamycin I and rifamycin W. The 360 MHz-NMR spectra of protorifamycin I and rifamycin W are shown in Fig. 2. The signals of the protons in the ansa chain were found at very similar chemical shifts in both spectra indicating no structural differences in the ansa part. Besides the signal of the aromatic C-3 proton at 7.5 ppm, a new singlet of an aromatic proton was found at 7.9 ppm in the spectrum of protorifamycin I. It is attributed to the proton at C-8 for the following reason: The chemical shifts for the C-8 proton and the C-6 hydroxyl group found in the NMR spectrum of protorifamycin I are exactly the same as were found earlier in the protostreptovaricins[5] with the hydroxyl in 6-position.

The $^{13}$C-NMR spectrum of protorifamycin I (data see Table 1) is in good agreement with the postulated structure. By comparison with the $^{13}$C-NMR spectra of rifamycin S[13~15] and rifamycin W

and with the aid of the off-resonance spectrum of protorifamycin I the carbons could be assigned (see Table 1). Compared to rifamycin W strong shift differences were found for some aromatic carbons of the chromophore due to the missing C-8 hydroxyl group, whereas there were no differences in the chemical shifts for the carbon atoms of the ansa chain. The most drastic differences were observed for the three carbon atoms C-7 to C-9 which were found at 133.0, 131.6 and 124.0 ppm in protorifamycin I compared to 119.2, 163.6 and 108.1 ppm, respectively, in rifamycin W. As in the $^{13}$C-NMR spectrum of rifamycin W the shifts for the hydroxymethyl group C-34a and the methine group C-28 were found at 64.3 ppm and 49.3 ppm, respectively. In the off-resonance spectrum of protorifamycin I the signal of C-34a forms a triplet, whereas the signal of C-28 was found to be a doublet.

These findings prove that protorifamycin I has the same ansa chain as rifamycin W. The possibility that protorifamycin I is identical with 3-demethylprotostreptovaricin III, bearing a hydroxyl group at C-28 instead of C-34a can thus be excluded. Furthermore, streptovaricin C, which also has a hydroxyl group at C-28, is cleaved by reaction with periodic acid, whereas protorifamycin I shows no reaction. Further support for the structure of protorifamycin I was obtained by reaction of protorifamycin I with methyl iodide in the presence of silver oxide to give 6-O-methylprotorifamycin I. The FD mass spectrum shows a molecular ion at *m/e* 653 and the O-methylchromophoric ions at *m/e* 244 and 272, which are 14 mass units heavier than in the mass spectrum of protorifamycin I. In the $^{13}$C-NMR spectrum (data see Table 1) the signal of an extra methyl group at 62.8 ppm is visible. The aromatic carbon atoms C-5 to C-10 have shifted again due to the changing of the hydroxyl group to a methoxyl group. A small upfield drift from 200.2 to 198.9 ppm was observed for the carbonyl C-11. This can be explained by the replacement of the hydrogen-bonded hydroxyl group by the methyl ether group. This is another argument for the hydroxyl group being in position 6 of the aromatic ring. With these findings the structure of protorifamycin I seems to be well established.

## Biological Activity

Protorifamycin I shows no activity against Gram-positive bacteria, Gram-negative bacteria and *Candida albicans* (MIC>64 $\mu$g/ml).

## Transformation Studies

Shake flasks (200-ml) with 40 ml of liquid complex medium 148 were inoculated with mycelium (60 mg dry weight) of the strains *Nocardia mediterranei* N813 (rifamycin B producer), 126 (rifamycin W producer from the Lepetit research laboratories) and W2800 (rifamycin W producer from the laboratories of Ciba-Geigy, provided by R. ROOS and T. SCHUPP) and fermented for 4 days at 28°C and 250 rpm. Then the mycelia were washed twice with 0.066 M phosphate buffer (pH 7) and resuspended in 25 ml of 0.066 M phosphate buffer (pH 7). The following incubations were carried out (50-ml shake flasks, 80 hours, 250 rpm, 28°C):

(1) Transformation assay:
5 ml mycelium-suspension (N813, 126, W2800) in phosphate buffer pH 7
0.5 ml protorifamycin I solution containing 6 mg/ml protorifamycin I in phosphate buffer pH 7
0.5 ml 50% glucose

(2) Control (*de novo* synthesis):
5 ml mycelium-suspension (N813, 126, W2800) in phosphate buffer pH 7
0.5 ml phosphate buffer pH 7
0.5 ml 50% glucose

(3) Control without mycelium:
as (1) but with 5 ml phosphate buffer pH 7 instead of mycelium-suspension

After incubation the culture filtrates were acidified (pH 2 with 2 N sulfuric acid), extracted with 1 ml of ethyl acetate and centrifuged and the ethyl acetate layer was assayed by TLC on pre-coated silica gel 60 F-254 plates (Merck) using 1-butanol - acetic acid - water (80: 20: 20); (Rf values: rifamycin B 0.67, rifamycin W 0.82, protorifamycin I 0.76) and chloroform - methanol (80: 20); (Rf values: rifamycin B 0.24, rifamycin W 0.32, protorifamycin I 0.35) as solvent systems. With all the three strains N813, 126 and W2800 a partial transformation of protorifamycin I to rifamycin W was observed. With N813 the formation of a small amount of rifamycin B from protorifamycin I could also be detected. These findings show clearly that protorifamycin I must be a biogenetic precursor for both rifamycin W and rifamycin B.

## Discussion

Protorifamycin I is the earliest precursor of the rifamycins described so far. As shown in the introduction it is not only a precursor of rifamycin W but it is also closely related to protostreptovaricin I, the earliest precursor of the streptovaricins known.

A common progenitor for both protorifamycin I and protostreptovaricin I could have one of the hypothetical structures proposed in Fig. 3. From the hypothetical "proansamycin B" (34a-deoxyprotorifamycin I or 3-demethylprotostreptovaricin I) protorifamycin I and protostreptovaricin I could be synthesized by simple one-step transformations. From the hypothetical "proansamycin A" (6,34a-dideoxyprotorifamycin I or 3-demethyl-6-deoxyprotostreptovaricin I) different two-step transformations would lead to protorifamycin I and protostreptovaricin I. If we assume that the methylation at C-3 for the ansamycins of the streptovaricin-type and the oxidation at C-34a for the ansamycins of the rifamycin-type take place only after the ring closure between C-5 and C-10 and after the formation of the peptide bond between the chromophore and the ansa-chain, the "proansamycins A and B" are the only two remaining possibilities for a common progenitor having the basic structure of all the naphthalenic ansamycins.

With our present knowledge we cannot decide which of these two hypothetical "proansamycins" is more likely to be the common progenitor for the naphthalenic ansamycins.

At the moment we have some preliminary spectroscopic evidence for the existence of further products lacking the hydroxyl group at C-8 of the naphthoquinone part of the molecule. These products are also components of the rifamycin complex produced by our strain *Nocardia mediterranei* F 1/24. Our future work will deal with the investigation of these compounds and possibly lead to the isolation of intermediates such as the postulated "proansamycins A and B".

Acknowledgement

The authors thank H. FRITZ and P. HUG (360 MHz-$^1$H-NMR spectra), H. FUHRER ($^{13}$C-NMR spectra), S. Moss (IR spectra), W. J. RICHTER (mass spectra) and J. A. L. AUDEN for reviewing the manuscript.

## References

1) WHITE, R. J.; E. MARTINELLI, G. G. GALLO, G. LANCINI & P. BEYNON: Rifamycin biosynthesis studied with $^{13}$C enriched precursors and carbon magnetic resonance. Nature 243: 273~277, 1973
2) WHITE, R. J.; E. MARTINELLI & G. LANCINI: Ansamycin biogenesis: Studies on a novel rifamycin isolated from a mutant strain of *Nocardia mediterranei*. Proc. Nat. Acad. Sci. 71: 3260~3264, 1974
3) MILAVETZ, B.; K. KAKINUMA, K. L. RINEHART, Jr., J. P. ROLLS & W. J. HAAK: Carbon-13 magnetic resonance spectroscopy and the biosynthesis of streptovaricin. J. Am. Chem. Soc. 95: 5793~5795, 1973
4) RINEHART, K. L., Jr.; F. J. ANTOSZ, P. V. DESHMUKH, K. KAKINUMA, P. K. MARTIN, B. I. MILAVETZ, K. SASAKI, T. R. WITTY, L. H. LI & F. REUSSER: Identification and preparation of damavaricins, biologically active precursors of streptovaricins. J. Antibiotics 29: 201~203, 1976

5) DESHMUKH, P. V.; K. KAKINUMA, J. J. AMEEL, K. L. RINEHART, Jr., P. F. WILEY & L. H. LI: Protostreptovaricins I-V. J. Am. Chem. Soc. 98: 870~872, 1976
6) WILLIAMS, T. H.: Naphthomycin, a novel ansamacrocyclic antimetabolite. Proton NMR spectra and structure elucidation using lanthanide shift reagent. J. Antibiotics 28: 85~86, 1975
7) WEHRLI, W.: Ansamycins: Chemistry, biosynthesis and biological activity. Topics in Current Chemistry. 72: 21~49, 1977, Springer-Verlag, Berlin, Heidelberg, 1977
8) MARTINELLI, E.; G. G. GALLO, P. ANTONINI & R. J. WHITE: Structure of rifamycin W: A novel ansamycin from a mutant of *Nocardia mediterranei*. Tetrahedron 30: 3087~3091, 1974
9) SCHUPP, T.: Genetik von *Nocardia mediterranei*. Dissertation Nr. 5153, Federal School of Technology, Switzerland, 1973
10) GHISALBA, O.: Untersuchungen über den Zusammenhang der Rifamycin-Chromophor-Biosynthese mit der Aromaten-Biosynthese über den Shikimisäure-Weg. Dissertation, University of Basle, Switzerland, 1978
11) ZERILLI, L. F.; M. LANDI, N. RIMORINI & G. G. GALLO: Spettri di massa delle rifamicine. Annali di Chimica 64: 199~206, 1974
12) ZERILLI, L. F.; M. LANDI, G. G. GALLO, K. H. MAURER & U. RAPP: Field desorption mass spectra of rifamycins. Biomed. Mass Spectrom. 2: 307~312, 1975
13) MARTINELLI, E.; R. J. WHITE, G. G. GALLO & P. J. BEYNON: Carbon-13 NMR spectrum of rifamycin S: A re-examination of the assignments with special reference to their biogenetic implication. Tetrahed. Lett. 1974: 1367~1368, 1974
14) MARTINELLI, E.; R. J. WHITE, G. G. GALLO & P. J. BEYNON: Carbon-13 NMR spectra of rifamycins. Tetrahedron 29: 3441~3448, 1973
15) FUHRER, H.: Zuordnung des $^{13}C$-NMR-Spektrums von Rifamycin S aufgrund der selektiven Protonen-Entkopplung. Helv. Chim. Acta 56: 2377~2386, 1973