A GENETIC APPROACH TO THE BIOSYNTHESIS OF THE RIFAMYCIN-CHROMOPHORE IN *NOCARDIA MEDITERRANEI*

IV. IDENTIFICATION OF 3-AMINO-5-HYDROXYBENZOIC ACID AS A DIRECT PRECURSOR OF THE SEVEN-CARBON AMINO STARTER-UNIT

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3-Amino-5-hydroxybenzoic acid was investigated for its ability to induce rifamycin biosynthesis in an appropriate mutant of *Nocardia mediterranei* and identified as a direct precursor of the seven-carbon amino starter-unit for the biosynthesis of ansamycins. A model for the biosynthesis of different types of ansamycins is presented and discussed.

As described in part III of this series of papers[1] we could isolate a very early aromatic ansamycin-precursor containing the seven-carbon amino starter-unit and three initial acetate/propionate-units of the ansa chain. This precursor was designated product P8/1-OG and identified as 2,6-dimethyl-3,5,7-trihydroxy-7-(3′-amino-5′-hydroxyphenyl)-2,4-heptadienoic acid. The structural comparison between product P8/1-OG and rifamycin S or rifamycin W clearly demonstrated a common biogenetic origin. 3-Amino-5-hydroxybenzoyl-coenzyme A was proposed as a starter-molecule for both the biosynthesis of product P8/1-OG and the biosynthesis of rifamycins (ansamycins).

In order to prove this hypothesis, supplementation studies and cosynthesis experiments were carried out using P^--strains[1] of *Nocardia mediterranei* and the transketolase$^-$-mutant *N. mediterranei* A8[2].

Experiments and Results

Synthesis of 3-Amino-5-hydroxybenzoic Acid

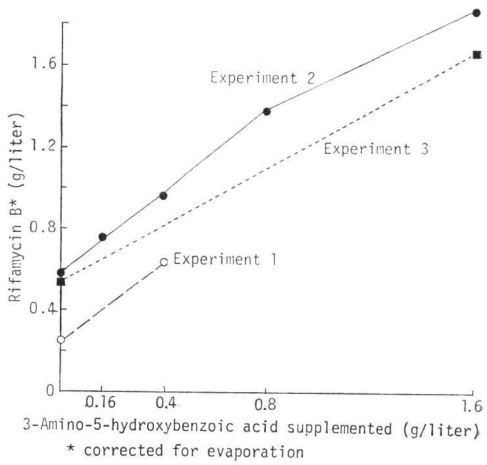
3-Amino-5-hydroxybenzoic acid is not commercially available. The compound was synthesized starting from 3,5-dinitrobenzoic acid according to the following method (modified method based on BRAY *et al.*[3] and BICKEL *et al.*[4]). A solution of 100 g 3,5-dinitrobenzoic acid (0.472 moles) in 750 ml of methanol and a solution of 100 g $NaHS \cdot H_2O$ (1.35 moles) in 1,500 ml of 65% methanol were combined and the reaction mixture was stirred for 20 minutes; 300 ml of 4 N HCl were then added, the mixture was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in 1,000 ml of methanol and the insoluble NaCl was separated by filtration and the filtrate again evaporated to dryness. This operation was repeated with 500 ml of acetone and 90 g of 3-amino-5-nitrobenzoic acid-hydrochloride was obtained. The product was tested for purity by TLC on silica gel plates using solvent system 1[2]. On the chromatogram (after air-drying) no 3,5-dinitrobenzoic acid (Rf 0.80, UV-254 nm), but only 3-amino-5-nitrobenzoic acid (Rf 0.85, orange) and traces of 3,5-diaminobenzoic acid (Rf 0.56, brown) were detected.

3-Amino-5-nitrobenzoic acid - hydrochloride (90 g, 0.413 moles) were suspended in 800 ml of pyridine and 170 ml of acetic anhydride ($\sim$1.5 moles) were added in small portions under shaking.

The mixture was stirred for two hours at 25°C and then evaporated to dryness under reduced pressure. The residue was crystallized from 96% ethanol at 4°C. 3-Acetamido-5-nitrobenzoic acid (69 g) were obtained as a dull yellow powder (m.p. 283~284°C; TLC pure: Rf 0.86, UV-254/366 nm). 3-Acetamido-5-nitrobenzoic acid (68 g, 0.304 moles) were suspended (partially dissolved) in 7.4 liters of methanol and 11.72 g HCl (1.5 equivalents) dissolved in 400 ml of methanol were added. Hydrogenation was then carried out for 10 hours at 25°C using 17 g of 10% Pd-C as a catalyst. After hydrogenation the catalyst was separated and the filtrate evaporated to dryness. 3-Acetamido-5-aminobenzoic acid-hydrochloride (64.7 g) was obtained as a dull grey powder (m.p.: >250°C, decomposes; TLC: Rf 0.80, brown, contains a trace of 3,5-diaminobenzoic acid).

3-Acetamido-5-aminobenzoic acid-hydrochloride (50 g, 0.212 moles) was dissolved in 1,000 ml of 2 N HCl and cooled to 0°C. $NaNO_2$ (17.4 g) in 250 ml of water were added in portions (within 5 minutes) and the mixture was stirred for 15 minutes at 0°C and for 15 minutes at 25°C. After addition of another 1,000 ml of 2 N HCl the reaction mixture was stirred again for another 30 minutes. The solution was then added in portions to 5 liters of boiling water and the diazo compound was hydrolyzed by boiling for 60 minutes. After addition of 350 g of NaOH the mixture was boiled for another 8 hours to hydrolyze the amide and then neutralized (pH 7) with concentrated HCl and evaporated to dryness under reduced pressure. To remove the NaCl the residue was stirred three times with portions of 2 liters of ethanol and the evaporated filtrates yielded 17.0 g of crude product (Na salt). HCl (7.3 g) in 750 ml of ethanol were added and again NaCl was separated by filtration. After evaporation 8.9 g of crude 3-amino-5-hydroxybenzoic acid-hydrochloride was obtained. The product was crystallized from ethyl acetate and 7.35 g of pure product was obtained (m.p. dec. 200°C; TLC: Rf 0.84~0.86, brown). The IR spectrum of our product was identical with the IR spectrum of a reference sample of 3-amino-5-hydroxybenzoic acid-hydrochloride obtained from KELLER-SCHIERLEIN (Federal School of Technology, Zürich). The ^{13}C-NMR data are in good agreement with the structure of 3-amino-5-hydroxybenzoic acid-hydrochloride: spectrum in DMSO-d_6 (δ values in ppm): C(1) 133.3, C(2) and C(6) 114.1, C(3) 133.0, C(4) 115.3, C(5) 158.3 and carboxyl-C(7) 166.1.

Fig. 1. Stimulation of the rifamycin B production by 3-amino-5-hydroxybenzoic acid in cultures of *Nocardia mediterranei* A8. (industrial fermentation medium 151b, 10 days, 28°C, 250 rpm)



Cosynthesis Experiments

Cosynthesis experiments were carried out with the strains *Nocardia mediterranei* A8 (transketolase$^-$-mutant, drastically reduced rifamycin-production)[2] and *Nocardia mediterranei* P14 (P^--mutant, no rifamycin-production but accumulation of product P8/1-OG)[1]. Procedure: shake flasks (200-ml) with 40 ml of liquid complex medium 148[2] were inoculated with mycelium (60 mg dry weight) and incubated for 4 days (28°C. 250 rpm). Three ml of these cultures were transferred into shake flasks (200-ml, one baffle) with 40 ml of industrial fermentation me-

dium 15lb[5] and fermented for 5 days (28°C, 250 rpm). Twenty ml of each of two cultures A8 and P14 were then mixed and fermented for another 5 days (28°C, 250 rpm). Three parallel experiments were carried out. The mixed cultures of the strains A8 and P14 in industrial fermentation medium 15lb showed a significant increase of the rifamycin B production to 1.6 g/liter (tested by the method described by PASQUALUCCI *et al.*[6] and modified by BRUGGISSER[7]) compared with pure control cultures of strain A8 (0.61 g/liter after 10 days) and strain P14 (none). The values are corrected for evaporation. Now the question arised, whether this cosynthesis was due to product P8/1-OG (accumulation product of strain P14 and other P^--strains)[1] or due to an earlier aromatic metabolite produced by P14 and utilized by A8. To answer this question supplementation studies with *Nocardia mediterranei* A8 and product P8/1-OG were carried out.

Supplementation Studies with Product P8/1-OG

The fermentation procedure for this experiment was analogous to that described above, but instead of mixing two different cultures after 5 days of fermentation in industrial fermentation medium 15lb, 2 g/liter of product P8/1-OG (lactone form) were added to a culture of *Nocardia mediterranei* A8 and fermented for another 5 days (three parallel experiments). The addition of product P8/1-OG to cultures of strain A8 showed no significant effect on the production of rifamycin B. The supplemented cultures had a rifamycin B titer of 0.66 g/liter compared with 0.61 g/liter for the unsupplemented control cultures (values corrected for evaporation).

These results show clearly that the observed cosynthesis in mixed cultures of the strains A8 and P14 is not due to the utilization of product P8/1-OG. Thus another aromatic metabolite produced by strain P14 must be responsible for the biosynthesis of rifamycin B by strain A8 in the mixed culture.

Supplementation Studies with 3-Amino-5-hydroxybenzoic Acid

Supplementation experiments were carried out with the strains *Nocardia mediterranei* A8 and N813 (high rifamycin B producer)[2].

Procedure: Shake flasks (200-ml) with 40 ml of liquid complex medium 148 were inoculated with mycelium (60 mg dry weight) and fermented for 4 days (28°C, 250 rpm). Then 3.5~4.0 ml of this culture were transferred into shake flasks (200-ml, one baffle) with 40 ml industrial fermentation medium 15lb supplemented with different concentrations of 3-amino-5-hydroxybenzoic acid (3-amino-5-hydroxybenzoic acid-hydrochloride was neutralized (pH 7) with NaOH and after sterile filtration added to the medium) and fermented for 10 days (28°C, 250 rpm). Two to four parallel experiments were carried out for each concentration.

A strong stimulation of the rifamycin B production by strain A8 was observed as an almost linear function of the supplemented amount of 3-amino-5-hydroxybenzoic acid in three independent sets of experiments (see Fig. 1). The amount of rifamycin B produced by strain A8 is increased from 0.22~0.56 g/liter (unsupplemented) to 1.65~1.85 g/liter when supplemented with 1.6 g/liter 3-amino-5-hydroxybenzoic acid (equivalent to 2 g/liter 3-amino-5-hydroxybenzoic acid-hydrochloride added).

This titer of 1.65~1.85 g/liter rifamycin B is almost identical with the 1.7~2.0 g/liter reached in the control cultures with *Nocardia mediterranei* N813. Thus with the addition of 3-amino-5-hydroxybenzoic acid the original production capacity of the parent strain N813 can be restored for strain A8.

An addition of 0.4~3.2 g/liter 3-amino-5-hydroxybenzoic acid to fermentations of high rifamycin B producing strains such as *Nocardia mediterranei* N813 did not lead to an increase of the rifamycin B production. This result is not surprising because in the case of N813 the seven-carbon amino unit

derived from the shikimate pathway is not a limiting factor as for strain A8, where due to the transketolase$^-$-mutation only traces of shikimate pathway products are synthesized.

Supplementation Studies with Different Substituted Benzoic Acids

A number of commercially available mono-, di- or trisubstituted benzoic acids (Fluka chemicals) were assayed in supplementation experiments analogous to that described for 3-amino-5-hydroxybenzoic acid. All the compounds were tested in a concentration of 2 g/liter fermentation medium 15lb (the compounds were neutralized (pH 7) with NaOH and after sterile filtration added to the medium). Two parallel experiments with *Nocardia mediterranei* A8 were carried out for each compound and after 10 days of fermentation the production of rifamycin B was tested by the photometric method[8,7] and by TLC on silica gel plates (Merck) using solvent system 1[2]. The following rifamycin B titers were observed (concentrations in brackets, corrected for evaporation): unsupplemented control cultures (0.54 g/liter rifamycin B), 3-aminobenzoic acid (0.06 g/liter), 3,5-diaminobenzoic acid (only traces on TLC, photometric test disturbed by diazonium compound), 3-hydroxybenzoic acid (0.06 g/liter), 2,3-dihydroxy benzoic acid (0.03 g/liter), 2,4-dihydroxybenzoic acid (0.34 g/liter), 2,5-dihydroxybenzoic acid (0.60 g/liter), 2,6-dihydroxybenzoic acid (0.73 g/liter), 3,4-dihydroxybenzoic acid (0.55 g/liter), 3,5-dihydroxybenzoic acid (0.32 g/liter), 3,4,5-trihydroxybenzoic acid (0.73 g/liter), 3-amino-4-hydroxybenzoic acid (0.15 g/liter), 5-amino-2-hydroxybenzoic acid (0.51 g/liter), 4-amino-2-hydroxybenzoic acid (0.02 g/liter) and 3-amino-5-hydroxybenzoic acid (1.65 ~ 1.85 g/liter).

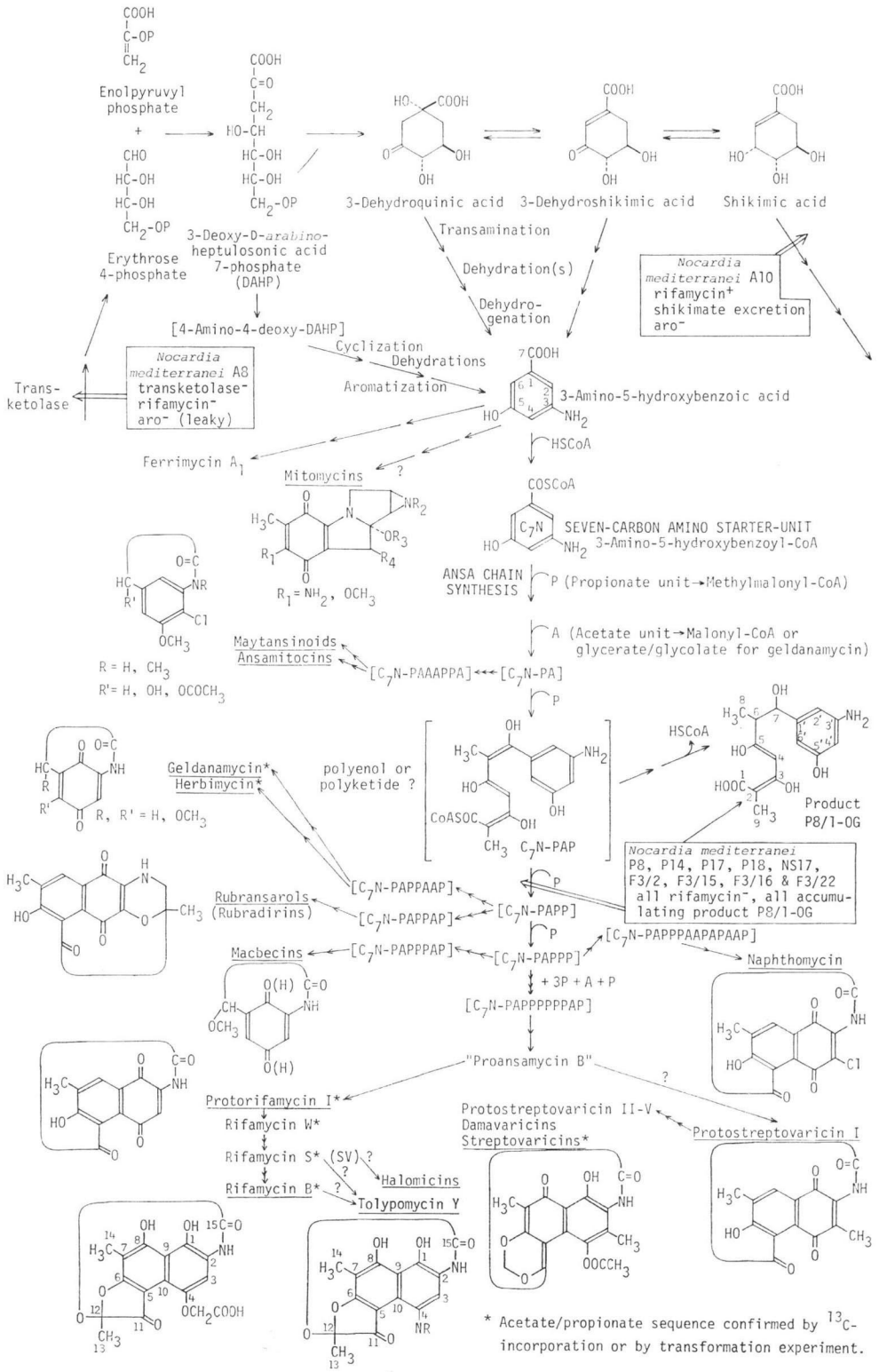
These results show clearly that only an addition of 3-amino-5-hydroxybenzoic acid can restore the rifamycin production capacity for strain A8. The other compounds show either only a very small increase of the rifamycin B production or no significant effect or in some cases even a drastic inhibition of the rifamycin production.

Discussion

The cosynthesis experiment with the strains *Nocardia mediterranei* A8 (transketolase$^-$, aro$^-$, rifamycin$^-$) and P14 (double mutant: 1. mutation: *aro*$^-$, shikimate accumulation. 2. mutation: rifamycin$^-$, P8/1-OG accumulation) demonstrated clearly that a metabolite excreted by strain P14 is utilized by strain A8 for the production of rifamycin B. The original rifamycin production capacity of the parent strain N813 can be restored for strain A8 by co-fermentation with strain P14. Product P8/1-OG (accumulation product of strain P14) in a supplementation experiment with *Nocardia mediterranei* A8 did not stimulate the production of rifamycin B and can therefore be excluded as the factor responsible for the increase of the rifamycin B production in the cosynthesis experiment. This is not surprising. From our model for the biosynthesis of ansamycins depicted in Fig. 2 it can be seen that the activated form of product P8/1-OG, its coenzyme A derivative, would act as a precursor in the biosynthesis of rifamycin B. The failure of product P8/1-OG to stimulate rifamycin production could be explained in different ways: P8/1-OG may not be able to penetrate into the cells of *Nocardia mediterranei* A8 or P8/1-OG can not be reactivated to P8/1-OG-CoA for further polyketide synthesis. Thus probably an earlier metabolite of strain P14 must be utilized by strain A8.

In the preceding paper[1], based on a biogenetic model for product P8/1-OG in analogy to the well-known incorporation pattern for ^{13}C-acetate and ^{13}C-propionate into rifamycins[8~11], we proposed 3-amino-5-hydroxybenzoic acid as its coenzyme A derivative as a seven-carbon amino starter-unit for ansamycin biosynthesis. 3-Amino-5-hydroxybenzoic acid could also be the metabolite in demand in our cosynthesis experiment. The supplementation studies with 3-amino-5-hydroxybenzoic acid and *Nocardia mediterranei* A8 demonstrate that this compound can indeed substitute for the seven-carbon amino unit. The original rifamycin production capacity of the parent strain N813 can be restored for strain A8 by supplementation with 3-amino-5-hydroxybenzoic acid. Our supplementation studies with

Fig. 2. Possible pathways for the biosynthesis of ansamycins starting from shikimate pathway intermediates *via* 3-amino-5-hydroxybenzoic acid as the direct precursor of the seven-carbon amino starter-unit.



13 other commercially available mono-, di- or trisubstituted benzoic acids did not lead to any positive results. This indicates that the activation of 3-amino-5-hydroxybenzoic acid with coenzyme A is a highly specific enzymatic reaction. It seems to be a strict requirement for the activation step that the right substituents (one hydroxyl and one amino group) are in the correct positions at C (3) and C (5) of benzoic acid.

With all the available biosynthetic data and by analyzing structural analogies we can depict a biogenetic model for all the known ansamycins (see Fig. 2). By chemical and biochemical analysis of *aro*$^-$-mutants we could demonstrate that the seven-carbon amino unit of rifamycin is derived from an intermediate of the shikimate pathway between 3-deoxy-D-arabinoheptulosonic acid 7-phosphate and shikimate[2,12].

However, the accurate branch point intermediate remains to be identified. Three possible pathways for the biosynthesis of 3-amino-5-hydroxybenzoic acid are shown in our model (Fig. 2) starting from 3-deoxy-D-arabinoheptulosonic acid 7-phosphate as proposed by HORNEMANN *et al.* for the mitomycins[13] or from 3-dehydroquinic acid or 3-dehydroshikimic acid. 3-Amino-5-hydroxybenzoic acid is the direct precursor for the seven-carbon amino starter-unit which can now be identified with 3-amino-5-hydroxybenzoyl-coenzyme A.

The antibiotics of the mitomycin-type[13,14] containing a seven-carbon amino unit similar to that of the ansamycins could be derived directly from 3-amino-5-hydroxybenzoic acid (the carboxyl group is reduced to a methyl group in one of the following biosynthetic steps). In the case of the antibiotic ferrimycin A_1[4], 3-amino-5-hydroxybenzoic acid itself is a structural element of the compound.

Starting with 3-amino-5-hydroxybenzoyl-CoA a polyketide chain (ansa chain) is built up by subsequent condensation with propionate and acetate units (*via* methylmalonyl-CoA and malonyl-CoA, respectively). Different biosynthetic branch points in the polyketide synthesis can be located by analyzing structural analogies of the ansamycin-types (ansa methyl groups indicate a propionate unit!) or by the known incorporation patterns for ^{13}C-acetate and ^{13}C-propionate (for detailed structures of the ansa chains see the cited references). A first ansamycin branch derived from the sequence C_7N-propionate-acetate (C_7N-PA) leads to the maytansinoids[15] and ansamitocins[16,17]. The sequence C_7N-PAP is excreted as its deactivated form (product P8/1-OG) by nine independent production$^-$-mutants of *Nocardia mediterranei*[1]. The following ansamycin-types are derived from polyketides behind the sequence C_7N-PAP: geldanamycin[18,19] and herbimycin[20], rubradirins (containing the ansamycin moieties rubransarol A or B)[21], macbecins[22], streptovaricins[24] *via* protostreptovaricins[25] and damavaricins[26], rifamycins[9~11] *via* proansamycin B[27] and protorifamycin I[5,28], halomicins[29,30] and tolypomycin Y[31].

The structural features of 3-amino-5-hydroxybenzoic acid are clearly visible in all these ansamycins. In all cases the nitrogen function and the oxygen function are both present in the meta-position to the carbon atom originating from the carboxy group of 3-amino-5-hydroxybenzoic acid (C(8) in the case of rifamycin). For tolypomycin Y and the halomicins the oxygen function at C (4) is replaced by a nitrogen function. The oxygen function originating from C (7) of 3-amino-5-hydroxybenzoic acid is lost in ansamitocins, maytansinoids (except colubrinol and colubrinol acetate), geldanamycin, rubransarols, naphthomycin, protorifamycin I and protostreptovaricins, but is still present in herbimycin and macbecins. For the naphthalenic ansamycins this oxygen function must be eliminated during the formation of the left aromatic ring. In later biosynthetic steps leading to the formation of rifamycins, halomicins, tolypomycin Y, damavaricins and streptovaricins, the oxygen at C (8) (rifamycin numbering) is again introduced (protorifamycin I→rifamycin W[5], protostreptovaricins→damavaricins and streptovaricin[25]). The oxygen function at C (1) of the chromophore (rifamycin numbering; originating from C (2) of 3-amino-5-hydroxybenzoic acid) which is present in most of the ansamycins listed above is still absent in maytansinoids and ansamitocins thus indicating that this oxygen must be absent in the seven-carbon amino starter-unit. In some of the ansamycins additional substituents such as chlorine, hydroxyl, methoxy or methyl are introduced into the chromophore in later biosynthetic steps (in positions corresponding to C (4) or C (6) of 3-amino-5-hydroxybenzoic acid).

Considering all these structural comparisons it seems to be very likely that 3-amino-5-hydroxyben-

zoic acid may act as a direct precursor for the seven-carbon amino starter-unit not only for rifamycins but also for the other ansamycin-types.

Note added in proof

After the preparation of this manuscript it came to our knowledge that KIBBY *et al.*[32] have carried out very similar experiments. They synthesized [carboxy ^{14}C]3-amino-5-hydroxybenzoic acid and showed that this labelled compound is incorporated into actamycin an ansamycin similar to naphthomycin. They come to the same conclusion that 3-amino-5-hydroxybenzoic acid is the key C_7N precursor which initiates ansa chain formation in the ansamycins. Thus with two different approaches the same biosynthetic picture is now described for two different ansamycins.

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References

1) GHISALBA, O.; H. FUHRER, W. J. RICHTER & S. MOSS: A genetic approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*. III. Isolation and identification of an early aromatic ansamycin-precursor containing the seven-carbon amino starter-unit and three initial acetate/propionate-units of the ansa chain. J. Antibiotics 34: 58~63, 1981
2) GHISALBA, O. & J. NÜESCH: A genetic approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*. I. Isolation and characterization of a pentose-excreting auxotrophic mutant of *Nocardia mediterranei* with drastically reduced rifamycin-production. J. Antibiotics 31: 202~214, 1978
3) BRAY, H. G.; W. V. THORPE & P. B. WOOD: The fate of certain organic acids and amides in the rabbit. 12. Aminohydroxybenzoic acids. Biochem. J. 48: 394~399, 1951
4) BICKEL, H.; P. MERTENS, V. PRELOG, J. SEIBL & A. WALSER: Stoffwechselprodukte von Mikroorganismen. 53. Ueber die Konstitution von Ferrimycin A_1. Tetrahedron, Suppl. 8, Part I: 171~179, 1966
5) GHISALBA, O.; P. TRAXLER & J. NÜESCH: Early intermediates in the biosynthesis of ansamycins. I. Isolation and identification of protorifamycin I. J. Antibiotics 31: 1124~1131, 1978
6) PASQUALUCCI, C. R.; A. VIGEVANI, P. RADAELLI & G. G. GALLO: Improved differential spectrophotometric determination of rifamycins. J. Pharm. Sci. 59: 685~687, 1970
7) BRUGGISSER, S.: Zur Biosynthese des Rifamycin-Chromophores. Dissertation Nr. 5435, Federal School of Technology, Switzerland, 1975
8) KARLSSON, A.; G. SARTORI & R. J. WHITE: Rifamycin biosynthesis: Further studies on origin of the ansa chain and chromophore. Eur. J. Biochem. 47: 251~256, 1974
9) 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
10) BRUFANI, M.; D. KLUEPFEL, G. C. LANCINI, J. LEITICH, A. S. MESENTSEV, V. PRELOG, F. P. SCHMOOK & P. SENSI: Uber die Biogenese des Rifamycin S. Helv. Chim. Acta 56: 2315~2323, 1973
11) WHITE, R. J.; E. MARTINELLI & G. C. LANCINI: Ansamycin biogenesis: Studies on a novel rifamycin isolated from a mutant strain of *Nocardia mediterranei*. Proc. Nat. Acad. Sci. U.S.A. 71: 3260~3264, 1974
12) GHISALBA, O. & J. NÜESCH: A genetic approach to the biosynthesis of the rifamycin-chromophore in *Nocardia mediterranei*. II. Isolation and characterization of a shikimate excreting auxotrophic mutant of *Nocardia mediterranei* with normal rifamycin-production. J. Antibiotics 31: 215~225, 1978
13) HORNEMANN, U.; J. H. EGGERT & D. P. HONOR: Role of D-[4-^{14}C]erythrose and [3-^{14}C]pyruvate in the biosynthesis of the meta-C-C_6-N unit of the mitomycin antibiotics in *Streptomyces verticillatus*. J. Chem. Soc., Chem. Comm. 1980: 11~13, 1980
14) BEZANSON, G. S. & L. C. VINING: Studies on the biosynthesis of mitomycin C by *Streptomyces verticillatus*. Canad. J. Biochem. 49: 911~918, 1971
15) KUPCHAN, S. M.; Y. KOMODA, A. R. BRANFMAN, A. T. SNEDEN, W. A. COURT, G. J. THOMAS, H. P. J. HINTZ, R. M. SMITH, A. KARIM, G. A. HOWIE, A. K. VERMA, Y. NAGAO, R. G. DAILEY, V. A. ZIMMERLY & W. C. SUMMER: The maytansinoids. Isolation, structural elucidation and chemical interrelation of

novel ansa macrolides. J. Org. Chem. 42: 2349~2357, 1977

16) HIGASHIDE, E.; M. ASAI, K. OOTSU, S. TANIDA, Y. KOZAI, T. HASEGAWA, T. KISHI, Y. SUGINO & M. YONEDA: Ansamitocin, a group of novel maytansinoid antibiotics with antitumour properties from *Nocardia*. Nature 270: 721~722, 1977

17) ASAI, M.; E. MIZUTA, M. IZAWA, K. HAIBARA & T. KISHI: Isolation, chemical characterization and structure of ansamitocin, a new antitumour ansamycin antibiotic. Tetrahedron 35: 1079~1085, 1979

18) SASAKI, K.; K. L. RINEHART, Jr., G. SLOMP, M. F. GROSTIC & E. C. OLSON: Geldanamycin. I. Structure assignment. J. Am. Chem. Soc. 92: 7591~7593, 1970

19) HABER, A.; R. D. JOHNSON & K. L. RINEHART, Jr.: Biosynthetic origin of the C_2 units of geldanamycin and distribution of label from D-(6-^{13}C)glucose. J. Am. Chem. Soc. 99: 3541~3544, 1977

20) ŌMURA, S.; A. NAKAGAWA & N. SADAKANE: Structure of herbimycin, a new ansamycin antibiotic. Tetrahedron Lett. 1979: 4323~4326, 1979

21) HOEKSEMA, H.; C. CHIDESTER, S. A. MIZSAK & L. BACZYNSKYJ: The chemistry of the rubradirins. I. The structures of rubransarols A and B. J. Antibiotics 31: 1067~1069, 1978

22) MUROI, M.; K. HAIBARA, M. ASAI & T. KISHI: The structures of macbecin I and II, new antitumour antibiotics. Tetrahedron Lett. 21: 309~312, 1980

23) BRUFANI, M.; L. CELLAI & W. KELLER-SCHIERLEIN: Degradation studies of naphthomycin. J. Antibiotics 32: 167~168, 1979

24) 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

25) DESHMUKH, P.; 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

26) 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

27) GHISALBA, O.; P. TRAXLER, H. FUHRER & W. J. RICHTER: Early intermediates in the biosynthesis of ansamycins. II. Isolation and identification of proansamycin B-M1 and protorifamycin I-M1. J. Antibiotics 32: 1267~1272, 1979

28) GHISALBA, O.; P. TRAXLER, H. FUHRER & W. J. RICHTER: Early intermediates in the biosynthesis of ansamycins. III. Isolation and identification of further 8-deoxyansamycins of the rifamycin-type. J. Antibiotics 33: 847~856, 1980

29) GANGULY, A. K.; S. SZMULEWICZ, O. Z. SARRE, D. GREEVES, J. MORTON & J. MCGLOTTEN: Structure of halomicin B. J. Chem. Soc., Chem. Comm. 1974: 395~396, 1974

30) GANGULY, A. K.; Y. T. LIU, O. Z. SARRE & S. SZMULEWICZ: Structures of halomicins A and C. J. Antibiotics 30: 625~627, 1977

31) KISHI, T.; S. HARADA, M. ASAI, M. MUROI & K. MIZUNO: Tolypomycin. II. Structures of tolyposamine and tolypomycin Y. Tetrahedron Lett. 1969: 97~100, 1969

32) KIBBY, J. J.; I. A. MCDONALD & R. W. RICKARDS: 3-Amino-5-hydroxybenzoic acid as a key intermediate in ansamycin and maytansinoid biosynthesis. J. Chem. Soc., Chem. Comm. 1980: 768~769, 1980