Journal of Natural Products
Vol. 49, No. 6, pp. 957-970, Nov-Dec 1986

STUDIES ON THE BIOSYNTHESIS OF ANTIBIOTICS[1]

HEINZ G. FLOSS,* PAUL J. KELLER, and JOHN M. BEALE

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

ABSTRACT.—In the framework of studies on the biosynthesis of several antibiotics, specifically asukamycin, acarbose, reductiomycin, actinorhodin, and granaticin, a variety of different approaches to biosynthetic problems are illustrated. These include multiple labeling with stable isotopes in conjunction with modern methods of nmr analysis, as illustrated by the development of a new triple-quantum 2D-INADEQUATE experiment for biosynthetic studies, the use of stereochemical probes, as illustrated by the synthesis and stereochemical analysis of chiral[^{13}C,^{2}H]malonate, and combinations of genetic and chemical approaches, as illustrated by examples of the production of new hybrid antibiotics by genetic engineering.

Studies on the biosynthesis of secondary metabolites, like antibiotics, have for some time occupied a prominent place in natural product research. The insights gained from such work can be used to design more efficient routes for the total synthesis of complex natural products. They can be used to generate, by various techniques such as false precursor feeding or mutasynthesis, analogs of the normal natural products that may have more desirable biological properties. They can aid in the optimization of procedures for the commercial production of useful natural products. Based on biosynthetic knowledge, genetic engineering methods may allow the construction of genetically new organisms that produce new compounds. Last, but not least, the insights gained from biosynthetic studies eventually may provide answers to the enigmatic question why nature elaborates such a bewildering variety of organic molecules for many of which no apparent function is known.

In the following review, we will discuss work in the field of antibiotic biosynthesis from our laboratory. The examples are chosen not only to illustrate the types of questions in which we are interested, but also the different kinds of approaches that can be used to answer them. These include both chemical and spectroscopic approaches as well as biological methods.

Let us start our journey with the antibiotic asukamycin (Figure 1), a metabolite of

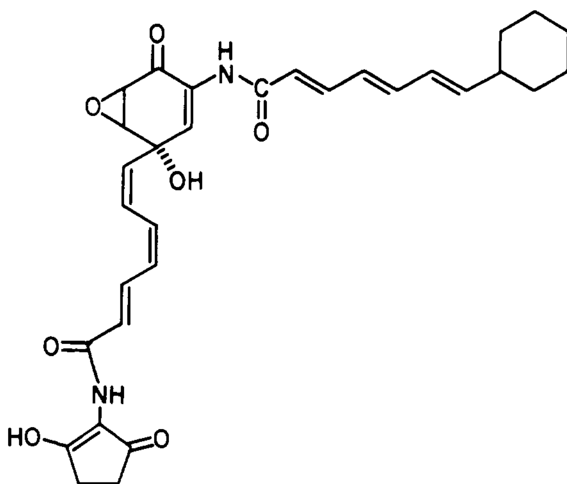

FIGURE 1. Structure of asukamycin

[1]Presented as a plenary lecture at the "Antibiotics, Molecular Biology, and Genetics" Symposium of the 27th Annual Meeting of the American Society of Pharmacognosy at the University of Michigan, Ann Arbor, Michigan, July 27-30, 1986.

Streptomyces nodosus subspecies *asukaensis* isolated by the group of Ōmura (1). We were interested in this compound because it contains several biosynthetically unusual moieties, namely, a cyclohexane ring, an epoxidized benzoquinol moiety, and an aminocyclopentandione portion. These three moieties are connected by short polyketide chains containing conjugated double bonds, some of which have *trans* and some of which have *cis* configuration. By feeding experiments with radioactive precursors, we were able to show that the cyclohexane ring and the adjacent carbon atom arise from shikimic acid via cyclohexanecarboxylic acid. The latter presumably represents the starter unit for one of the polyketide chains. Work is currently underway to define the sequence of steps involved in the conversion of shikimic acid to the completely saturated cyclohexane ring structure.

The six-membered ring in the center of the molecule carries a nitrogen and an additional carbon substituent in a 1,3(*meta*) arrangement. This structural element resembles the *m*-C_7N unit found in a number of different antibiotics, for example, the mitomycins, the ansamycins, and the ansamitocins (Figure 2). As demonstrated in a

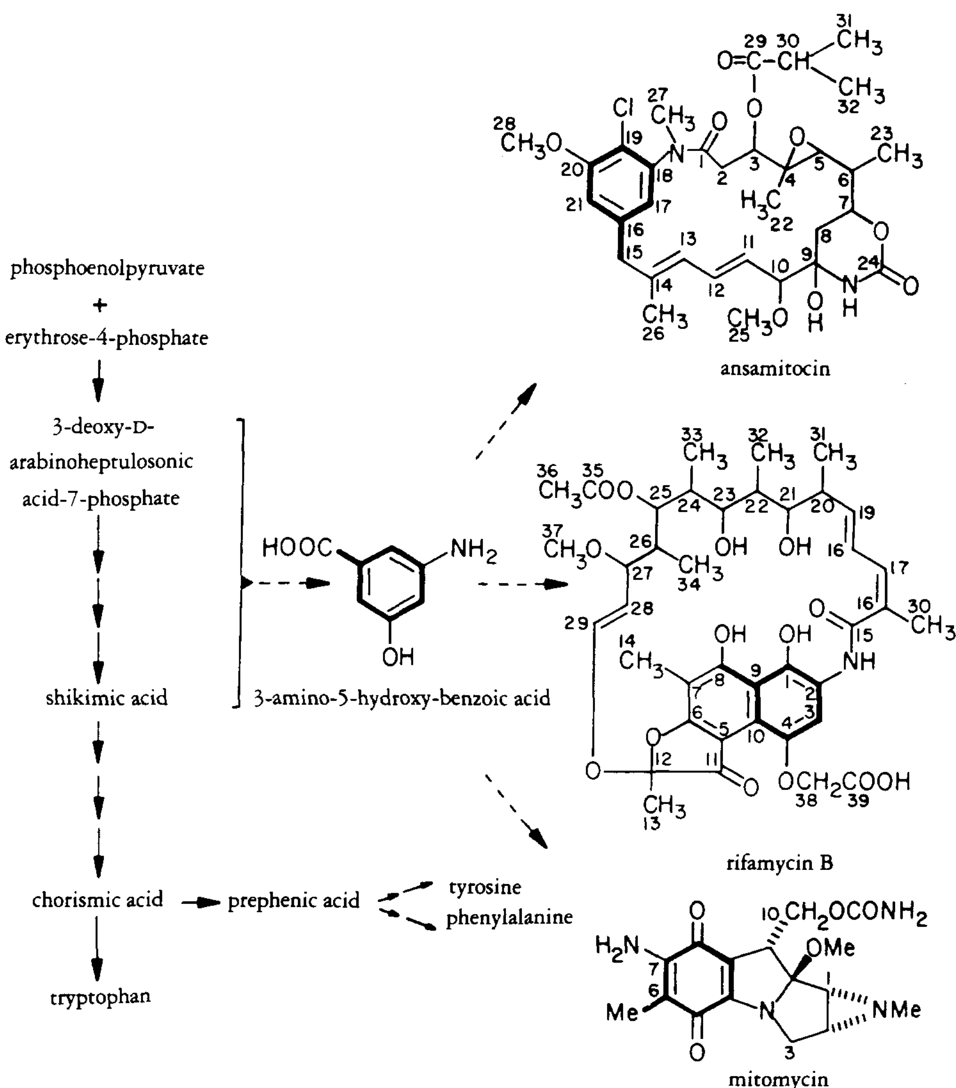

FIGURE 2. Origin of C_7N units in rifamycin, mitomycin, and ansamitocins

number of cases, this C_7N unit is in some way derived via the shikimate pathway, although shikimate itself is not a precursor, and 3-amino-5-hydroxybenzoic acid has been established as an intermediate (2-7). From our own work we can add two more examples of C_7N units derived from 3-amino-5-hydroxybenzoic acid. The compound, labeled with ^{13}C in the carboxyl group, was efficiently incorporated into the ansamycins ansatrienin (48% enrichment at C-17) and naphthomycin B (64% enrichment at C-8) (8). These compounds represent classical ansamycins in which the C_7N unit is present as a benzoquinone or as a naphthoquinone ring, respectively. A different kind of C_7N unit is represented by the aminocyclitol valienamine, first isolated from the antibiotic validamycin (9), which more recently has been found as a component of the α-glycosidase inhibitor acarbose (10) (Figure 3). It had been suggested (2) that valienamine is of the

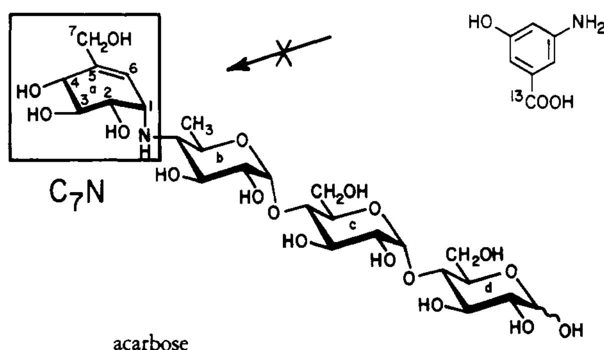

FIGURE 3. Structure of acarbose

same biosynthetic origin as the C_7N units in the mitomycins and rifamycins. However, a feeding experiment with 3-amino-5-hydroxy-[7-^{13}C]benzoic acid, carried out in collaboration with the group of Professor Pape in Germany, showed no incorporation into acarbose (11). In an additional feeding experiment with [U-$^{13}C_3$]glycerol we probed whether the valienamine moiety of acarbose is at all related in its origin to the shikimate pathway. The rationale for this type of experiment is that the proffered labeled glycerol will be diluted in the cell with large quantities of ^{12}C material. Hence, whenever the bond between two carbons of the labeled precursor is broken, the ^{13}C-^{13}C coupling is lost because of the large dilution with ^{12}C. Therefore, analysis of the ^{13}C-^{13}C coupling patterns in the product indicates which assemblies of carbon atoms have been incorporated intact from the added precursor, revealing the metabolic pathways leading from precursor to product. Experimentally, the analysis of these coupling patterns can sometimes be quite challenging. It is greatly simplified by the use of multiple quantum 2D-nmr techniques, which selectively identify assemblies of connected ^{13}C nuclei. In this particular case we used both the 2D-INADEQUATE (12) and a zero quantum coherence experiment (13), as well as a triple quantum version of the 2D-INADEQUATE experiment, which we developed (14). In this last experiment, unique assemblies of three coupled ^{13}C nuclei are identified by their common triple quantum coherence frequency. Part of the triple quantum 2D-INADEQUATE spectrum of acarbose derived from [U-$^{13}C_3$]glycerol is shown in Figure 4. The predominant coupling pattern established for the [U-$^{13}C_3$]glycerol-derived acarbose is shown in Figure 5. The two glucose units are not labeled, because they are derived by intact transfer of maltose, which, in unlabeled form, is a constituent of the culture medium. The 6-deoxyhexose moiety is derived directly from a hexose phosphate that in turn arises from two trioses. The coupling pattern in the valienamine moiety is different from that predicted for formation via the shikimate pathway but consistent with formation by cyclization of a heptulose

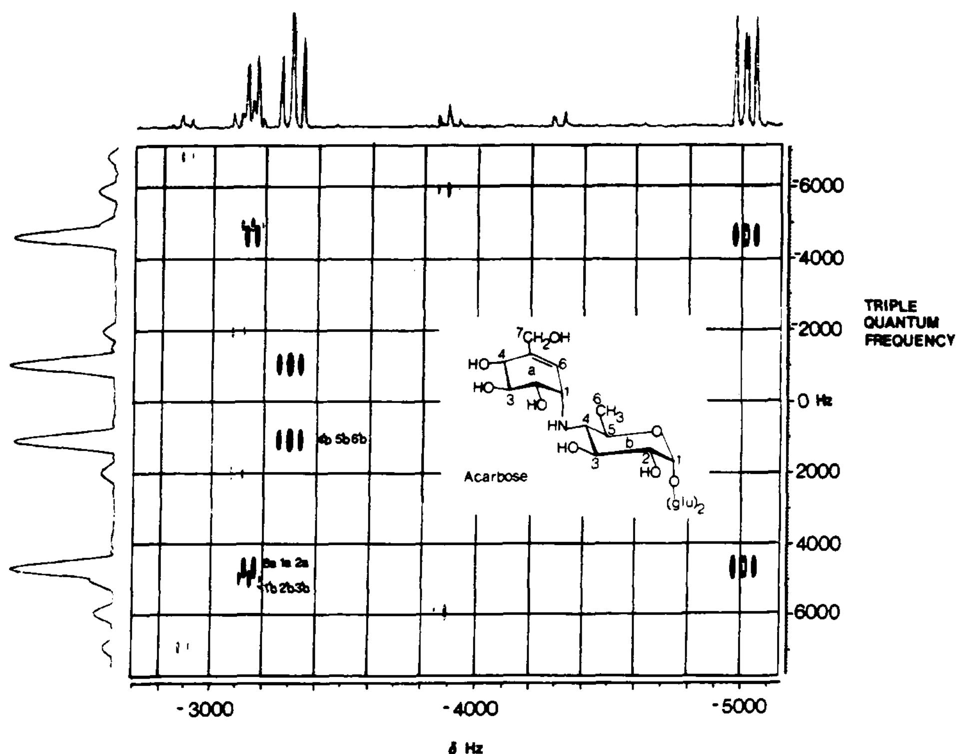

FIGURE 4. Part of the triple-quantum 2D-INADEQUATE spectrum of acarbose biosynthesized from [U-$^{13}C_3$]glycerol

phosphate, which in turn arises by successive transfer of two carbon units, catalyzed by transketolase, to a triose phosphate. Such a pathway (Figure 6) is supported also by additional feeding experiments carried out in Pape's laboratory (15).

Returning now to the biosynthesis of asukamycin, we were even more surprised to find that 3-amino-5-hydroxy-[7-^{13}C]benzoic acid was also not at all incorporated into this compound. Likewise, experiments in collaboration with the group of Professor Zeeck in Göttingen showed that 3-amino-5-hydroxy-[7-^{13}C]benzoic acid was also not incorporated into the closely related antibiotic manumycin nor was this compound labeled by 3-amino-[7-^{13}C]benzoic acid (16), the precursor of the C_7N unit in pactamycin (17). Feeding experiments on asukamycin with variously labeled acetate samples gave the incorporation pattern shown in Figure 7, indicating the incorporation of two molecules of acetate in a "tail-to-tail" fashion. This is suggestive of the origin of part of the C_7N unit from a Krebs cycle dicarboxylic acid. The same pattern was seen in manumycin. Two of the three remaining carbon atoms are derived from glycerol by a pathway other than via acetate, as was shown in a feeding experiment with [U-$^{13}C_3$]glycerol. The origin of the remaining carbon atom is at the moment unknown, but it is apparently not derived from the methyl group of methionine. From the results

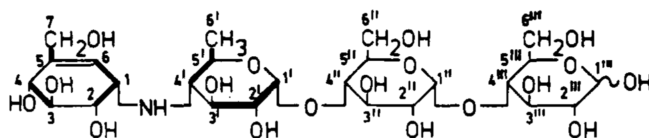

FIGURE 5. Predominant ^{13}C-^{13}C coupling pattern in acarbose derived from [U-$^{13}C_3$]glycerol

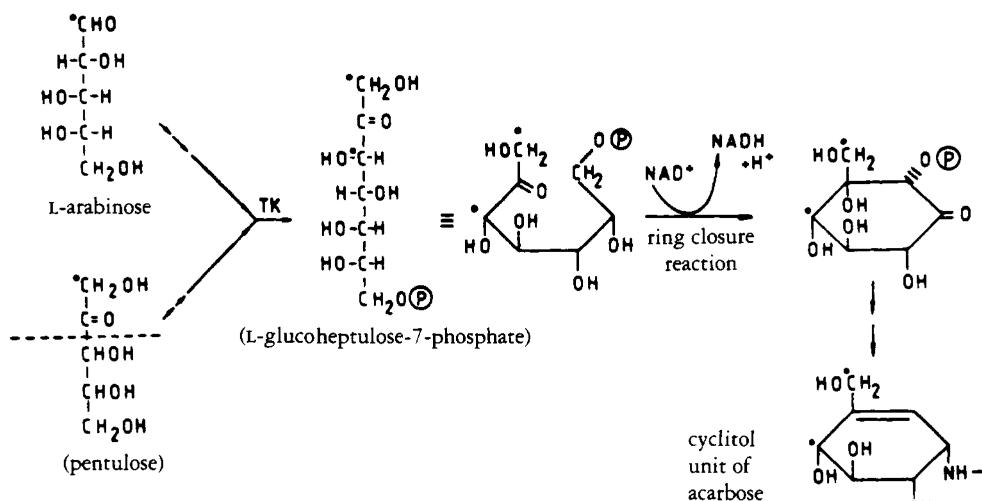

FIGURE 6. Metabolic pathway leading to the formation of the valienamine moiety of acarbose

on asukamycin and acarbose, it is clear that the various C_7N units found in natural products can have widely different biosynthetic origins.

The third structural element of interest in asukamycin, the aminocyclopentanedione moiety, is also found in a few other antibiotics, most of them of very recent vintage. These include manumycin (16), reductiomycin (18, 19), moenomycin A (20), senacarcin A (21), and a few others. Inspection of the aminocyclopentanedione moiety immediately reveals that it contains the entire framework of 5-aminolevulinic acid. Since aminolevulinic acid in microorganisms is formed by condensation of succinyl CoA and glycine with loss of the glycine carboxyl group, we tested the incorporation of ^{14}C-labeled glycine and succinic acid into asukamycin. Whereas the latter was a poor precursor, probably because of extensive Krebs cycle activity in the organism, glycine was effectively incorporated, giving specific incorporations of more than 20%. C-2 of glycine was incorporated almost nine times better than C-1. Intact incorporation of C-2 and the nitrogen of glycine into asukamycin was established in an experiment with [2-^{13}C,^{15}N]glycine. The ^{13}C-nmr spectrum of the resulting asukamycin showed a pronounced doublet flanking the non-coupled natural abundance singlet for the signal for C-2″ due to coupling of ^{13}C to the attached ^{15}N. Finally, 5-amino-[5-^{14}C]levulinic acid was fed and gave modest specific incorporations of up to 2%. The compound was rather unstable under the conditions of the experiment and decomposed with the formation of copious amounts of black pigments. Based on these results, we proposed that the aminocyclopentanedione moiety of asukamycin arises by a pyridoxal phosphate-catalyzed intramolecular cyclization of 5-aminolevulinic acid as shown in Figure 8 (22).

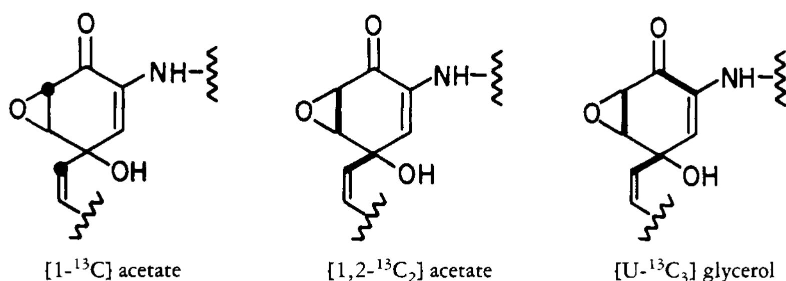

FIGURE 7. ^{13}C-^{13}C labeling and coupling pattern in the C_7N unit of asukamycin and manumycin derived from acetate and glycerol

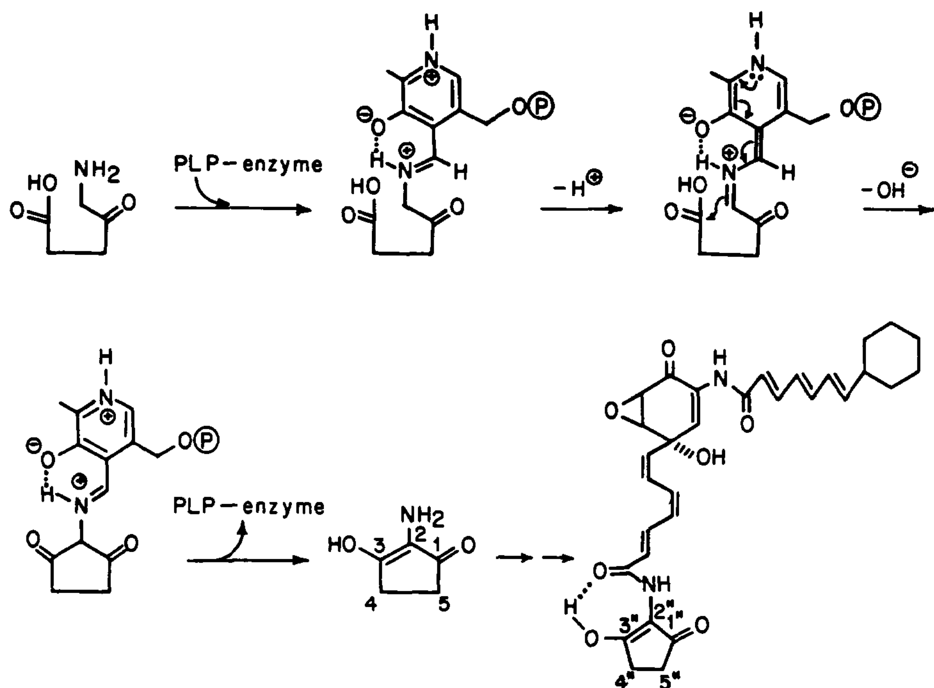

FIGURE 8. Proposed mechanism for the formation of the 2-amino-cyclopentenol-3-one moiety of asukamycin by intramolecular cyclization of 5-aminolevulinic acid

Intact incorporation of 5-aminolevulinic acid into an aminocyclopentanedione moiety was demonstrated unequivocally by feeding 5-amino-[4,5-$^{13}C_2$]levulinic acid to the organism producing the antibiotic reductiomycin (Figure 9). ^{13}C-nmr analysis indicated the expected enrichment of carbon atoms 1, 2, and 3 of the aminocyclopentanedione moiety, C-2 being twice as enriched as the other two carbons, and coupling of C-2 to C-1 in half the molecules and to C-3 in the other half (23).

The origin of the other half of the reductiomycin molecule could be traced to the shikimate pathway. We originally hypothesized that all nine carbon atoms could arise in their entirety from a molecule of tyrosine by ring cleavage followed by a Baeyer-Villiger oxidation. However, a feeding experiment with [1,2-$^{13}C_2$]acetate revealed that the acetoxy group is derived directly from acetate. To establish the origin of the remaining seven carbon atoms we again fed [U-$^{13}C_3$]glycerol. The analysis of the resulting reductiomycin by 1D- and 2D-nmr spectroscopy revealed the presence of two distinct

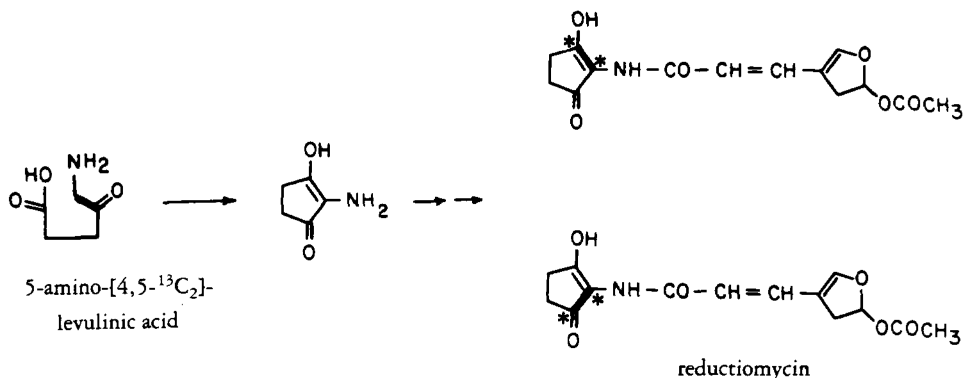

FIGURE 9. Structure of reductiomycin and its formation from 5-amino-[4,5-$^{13}C_2$]levulinic acid

coupling patterns in the seven-carbon moiety in question. These two patterns, **a** and **b** in Figure 10, can arise by ring cleavage of a symmetrical derivative of shikimic acid, for example, para-hydroxybenzoic acid. Cleavage at **a** gives rise to one pattern; cleavage at **b** to the other. The precursor role of hydroxybenzoic acid was immediately confirmed by feeding the compound labeled with ^{13}C in the carboxyl group and observing a very high ^{13}C-enrichment [64%] in the appropriate carbon in reductiomycin (23). We suspect that the actual substrate for the aromatic ring cleavage may be hydroxybenzaldehyde and are in the process of testing this idea.

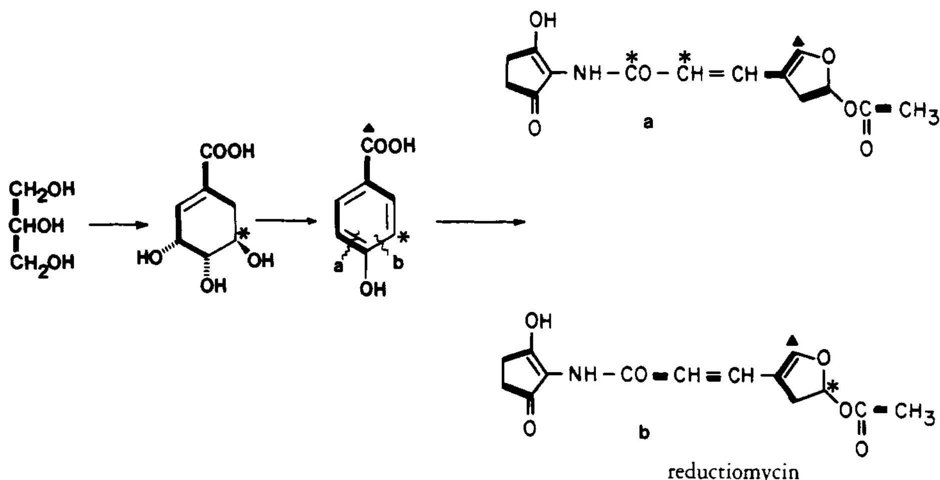

FIGURE 10. Origin of the dihydrofuranylacrylic acid moiety of reductiomycin from [U-$^{13}C_3$]glycerol via the shikimate pathway

Returning to the biosynthesis of asukamycin, we are left with two short polyene chains that connect the three cyclic moieties. As expected, the acetate feeding experiments showed these to be derived from acetate/malonate units via the polyketide pathway. As can be seen, in this particular case some of the double bonds have *cis*- and some have *trans*-geometry, raising the questions whether both are formed independently or whether one arises by isomerization of the other. To investigate this and other stereochemical questions of polyketide biosynthesis, we decided to prepare a chirally labeled precursor as a probe. Malonyl coenzyme A, the actual substrate used by polyketide synthase to add chain extension units, which had been prepared earlier in chirally labeled form by Cornforth and co-workers (24) was not suitable, because it can be used only in cell-free systems. We therefore decided to synthesize the *R* and the *S* isomer of [1-$^{13}C_1$,2-$^{2}H_1$]malonic acid, the first example of a chiral version of a molecule of the type Caabb (25). There is an obvious technical problem with this, because the methylene hydrogens of malonic acid are quite acidic and exchange fairly rapidly even at room temperature. The half-life for exchange at 30° ranges from 1.43 min at pH 3 to 216 min at pH 9. Clearly, the formation of malonic acid has to be carried out at a fairly alkaline pH, and the synthesis has to be structured such that virtually no final purification is necessary. We use the synthetic route shown in Figure 11, which generates the appropriately stereospecifically labeled malic acids that are then oxidized under controlled conditions to give dipotassium malonate. The final oxidation is carried out at 0° and work up involves mere removal of MnO_2 by filtration to give a solution of pH 8.5 which can be used directly for biological experiments or can be lyophilized. Under these oxidation conditions, the yield is about 20% and the malonate shows less than 10% exchange. Hence, the samples should contain at least 80% enantiomeric excess of one stereoisomer.

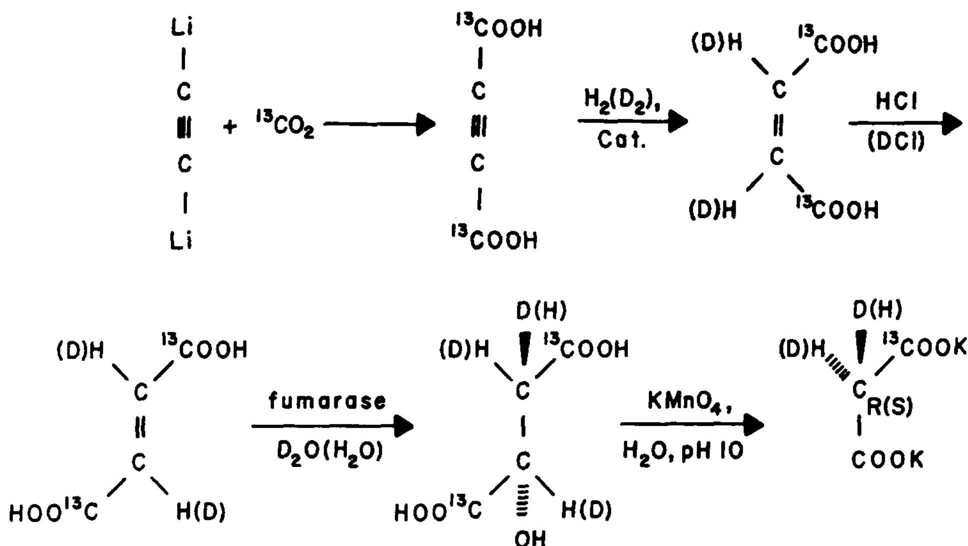

FIGURE 11. Synthesis of *R*- and *S*-potassium [1-$^{13}C_1$,2-$^2H_1$]-malonate

To prove that the chiral malonate is indeed optically active, we resorted to nmr analysis of a derivative with a chiral auxiliary. The reaction sequence used for the derivatization is shown in Figure 12. A considerable degree of exchange, and consequently racemization, is encountered during the acidification necessary to prepare the ester. Attempts to circumvent this by direct reduction of dipotassium malonate proved unsuccessful. The mixture of the two species of the mandelate monoester, containing also some diprotio species resulting from cleavage of the C-D bond by exchange, and some material of opposite configuration, resulting from C-H bond cleavage by exchange, was analyzed by ^{1}H-nmr spectroscopy. The spectra are shown in Figure 13. Observation with simultaneous deuterium and ^{13}C broadband decoupling (Figure 13, Panel 1) gives three signals from each of the two samples. These are the center of an AB system from the diprotio species and one singlet each from the 2*R* and the 2*S* monodeutero species. Upon removal of the ^{13}C broadband decoupling each of these signals splits into two with considerable overlap between them (Figure 13, Panel 2). The two opposite stereoisomers still cannot be distinguished. However, if the ^{1}H spectrum is observed with broadband deuterium decoupling and single frequency decoupling of

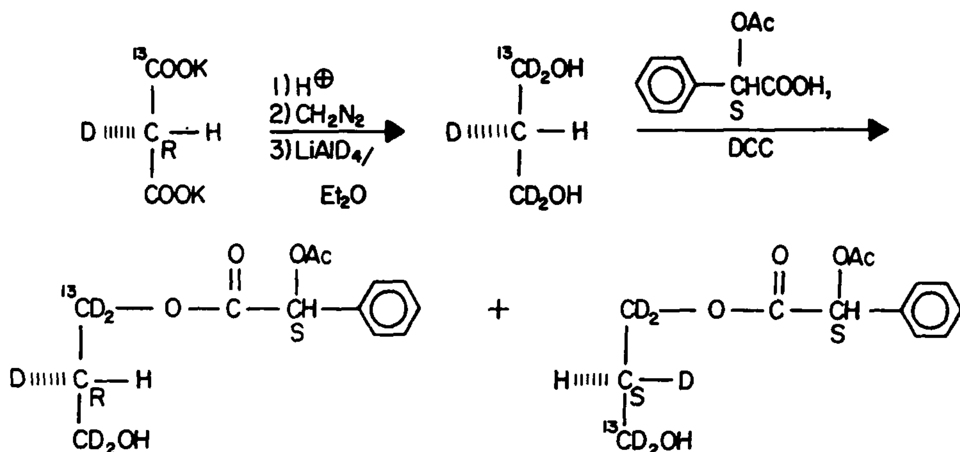

FIGURE 12. Derivatization of chiral malonate for configurational analysis by nmr

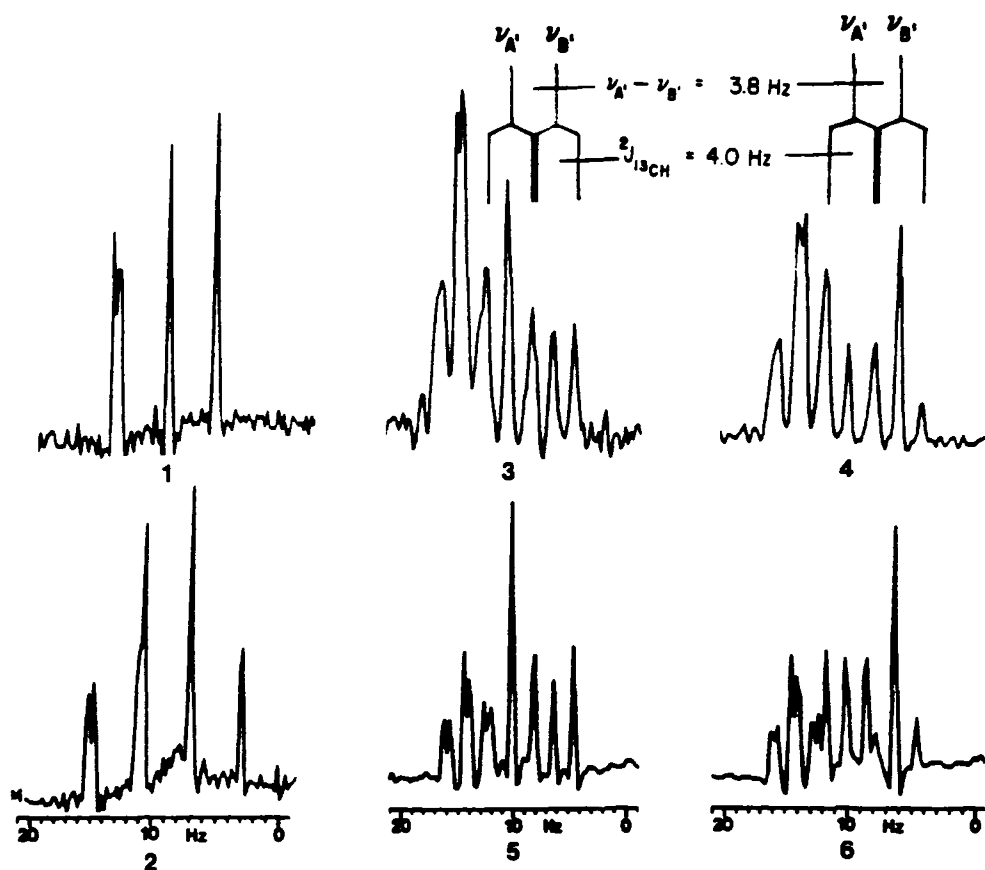

FIGURE 13. ^{1}H-nmr signal for the C-2 protons of [1,1,2,3,3-$^2H_5$]propane-1,3-diol mono-*O*-acetyl-D-mandelate observed with broad-band deuterium decoupling. (1) Product from (*R*)- or (*S*)-malonate with broad-band ^{13}C decoupling; (2) product from (*R*)- or (*S*)-malonate without ^{13}C decoupling; (3) product from (*S*)-malonate with selective ^{13}C decoupling of upfield (57.96 ppm) resonance; (4) product from (*S*)-malonate with selective ^{13}C decoupling of downfield (62.05 ppm) resonance; (5) product from (*R*)-malonate with selective ^{13}C decoupling of downfield resonance; (6) product from (*R*)-malonate with selective ^{13}C decoupling of upfield resonance

one of the ^{13}C signals present, one of the singlets from the monodeutero species splits predominantly into a doublet; whereas, the other predominantly remains a singlet. Thus, upon irradiation of the upfield ^{13}C signals in the products from the *S* malonate (Figure 13, Panel 3) or the downfield ^{13}C resonance in the products from *R* malonate (Figure 13, Panel 5), the A′ proton signal shows more singlet than doublet and the B′ signal shows more doublet than singlet. The reverse is seen upon decoupling the downfield resonance in the material from *S* malonate (Figure 13, Panel 4) and the upfield resonance in the product from *R* malonate. The ratio of the intensity of the singlet species for A′ versus B′ indicates the degree of enantiomeric excess. In both samples the analysis established approximately 34% e.e. The optical purity correlates well with the degree of exchange encountered during the derivatization as indicated by the diprotio species. This analysis, therefore, shows that this material is optically active and that the original malonate in all likelihood has a high degree of optical purity (25). With this material at hand, one can now attempt to establish the stereochemical course of various reactions involved in the biosynthesis of polyketides, such as asukamycin. This will still require a fair amount of development to establish conditions under which the stereochemical information is not completely obliterated by exchange during the course

of the experiment. The general principle of analysis is illustrated in Figure 14. It is based on the appearance of ^{13}C and deuterium in either the same molecules or different molecules, depending on the reaction stereochemistry, which can be determined either by mass spectrometry or nmr spectroscopy. Work is progress in our laboratory to implement such an analysis.[2]

Previous examples have focused on various chemical and spectroscopic methods for the analysis of biosynthetic pathways. We would like to conclude with an example of a more biological approach which resulted from a cooperation between chemists and molecular geneticists. Our laboratory has for some time studied the biosynthesis of isochromanequinone antibiotics of the type exemplified by dihydrogranaticin or ac-

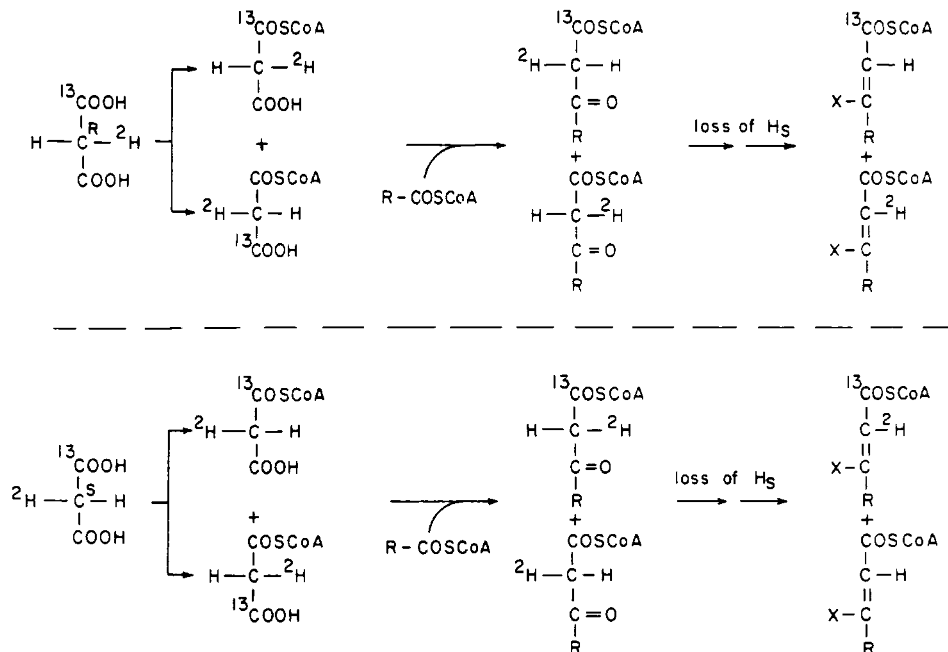

FIGURE 14. General approach for the stereochemical analysis of polyketide biosynthesis with (*R*)- and (*S*)-[1-$^{13}C_1$,2-$^2H_1$]malonate

tinorhodin (Figure 15). It will be noted that in addition to the constitutional differences, the two compounds also represent opposite enantiomeric series at the two chiral centers in the pyran ring. In a series of feeding experiments, the polyketide origin of the benzoisochromanequinone system for both actinorhodin (26) and granaticin (27) was established, as was the origin of the remaining six carbon atoms of granaticin from glucose (Figure 16) (27). In addition, many mechanistic details of granaticin and dihydrogranaticin biosynthesis were elucidated by the kinds of tracer techniques described in the previous sections (27, 28). Concurrently, the laboratory of Professor D.A. Hopwood at the John Innes Institute in England was involved in extensive genetic studies on *Streptomyces coelicolor*, the producer of actinorhodin. Rudd and Hopwood (29) prepared a large number of mutants of *S. coelicolor* that were blocked in the biosynthesis of actinorhodin as evidenced by the appearance of colorless colonies. A cosynthesis assay, growing two different mutants next to each other on a Petri dish and observing actinorhodin formation in one colony where it borders another, allowed them to group these mutants into several classes and to order these classes according to the position of

[2]NOTE ADDED IN PROOF: Jordan and co-workers (33) have recently also reported the synthesis of chiral malonate and its use in the biosynthesis of fatty acids.

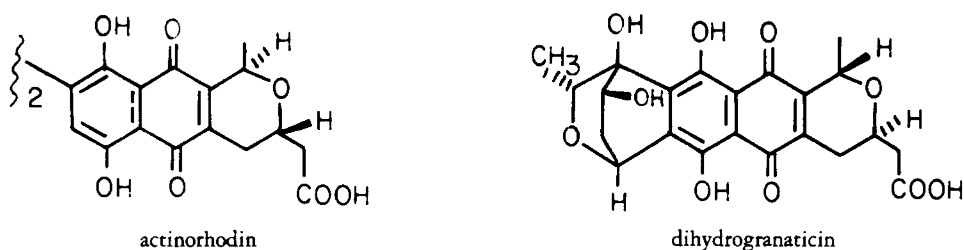

FIGURE 15. Structure of actinorhodin and dihydrogranaticin

the block in the biosynthetic pathway (Figure 17). We then analyzed representative members of these mutant classes to isolate, based on a modification of the cosynthesis assay, the various intermediates accumulated in the mutants. The isolated compounds, whose structures were determined, were a shunt product from mutant B_{40}, an intermediate from mutant B_{17} for which only a tentative structure has been established so far, an intermediate from mutant B_1, and kalafungin, also an intermediate from mutand B_{135} (Figure 18). The latter two mutants are both members of class V, which is obviously not homogeneous. The structures of these compounds together with additional information from the tracer studies allowed us to propose a reasonable, albeit hypothetical, pathway for the biosynthesis of actinorhodin (Figure 18) (30).

As this work was in progress, our geneticist colleagues continued their investigations. Mapping of the actinorhodin biosynthetic genes showed that they were all clustered in a narrow region of the *S. coelicolor* chromosome. This made it possible to clone the entire actinorhodin biosynthetic gene cluster (31). Initially, the 30 kb size fraction of partially digested *S. coelicolor* wild-type DNA was ligated into a suitable low copy number plasmid, which was used to transform one of the *act* mutants. Two transformants were isolated that were able to produce actinorhodin again. The plasmids reisolated from these two transformants contained DNA inserts that, between them, accounted for all the actinorhodin biosynthetic genes. By further subcloning, they were able to construct a plasmid carrying the entire actinorhodin biosynthetic gene sequence. This plasmid was capable of curing all the *act* mutant classes. Further evidence that it contained all the actinorhodin biosynthetic genes comes from the fact that transformation into a heterologous host, *Streptomyces parvulus*, a nonproducer of actinorhodin, resulted in a transformant producing actinorhodin (31). This accomplishment represents a milestone in genetic engineering, as it represents the first case of the cloning of the genes for an entire biosynthetic pathway and their expression in an heterologous host, leading to the production of a compound of small molecular weight. In a further

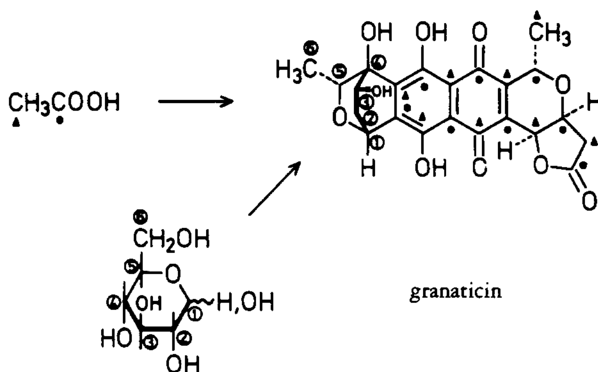

FIGURE 16. Biosynthetic origin of granaticin

Classes of act mutants and their phenotype

Mutant class	Type strain	No. of mutants in class	Diffusible pigment	Antibiotic activity against S. aureus	Cosynthesis as convertor	Cosynthesis as secretor
I	B78	13	-	-	IV,V,VI,VII	-
II	2377	26	-	-	-	-
III	B41	7	Red	-	IV,V,VI,VII	-
*VII	B40	2	Light golden brown	(+)	?,IV,V,VI	I,III
IV	B17	5	Reddish brown	-	V,VI	I,III
VI	B22	2	Light brown	-	V	I,III,IV
V	B1	21	Brown	+	-	I,III,IV,VI

*Mutants of class VII produced small quantities of actinorhodin which is presumed to be responsible for the limited inhibition of Staph. aureus. This also made it difficult to be sure of cosynthesis reactions, but it is probably that class VII mutants are cross-fed by mutants of IV, V and VI.

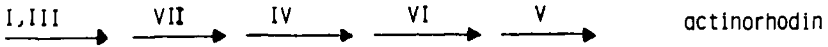

FIGURE 17. Classes of *act* mutants and their order in the biosynthetic pathway

collaboration between Hopwood's laboratory, the group of Ōmura, and our laboratory, this cloning technique was used to generate modified structures of antibiotics in the isochromanequinone series. Transformation of a *Streptomyces* species producing the antibiotic medermycin (Figure 19) by a plasmid containing part of the actinorhodin gene cluster led to the production of a new antibiotic, mederrhodin A, which had the structure of medermycin but contained the additional hydroxyl group which is characteristic

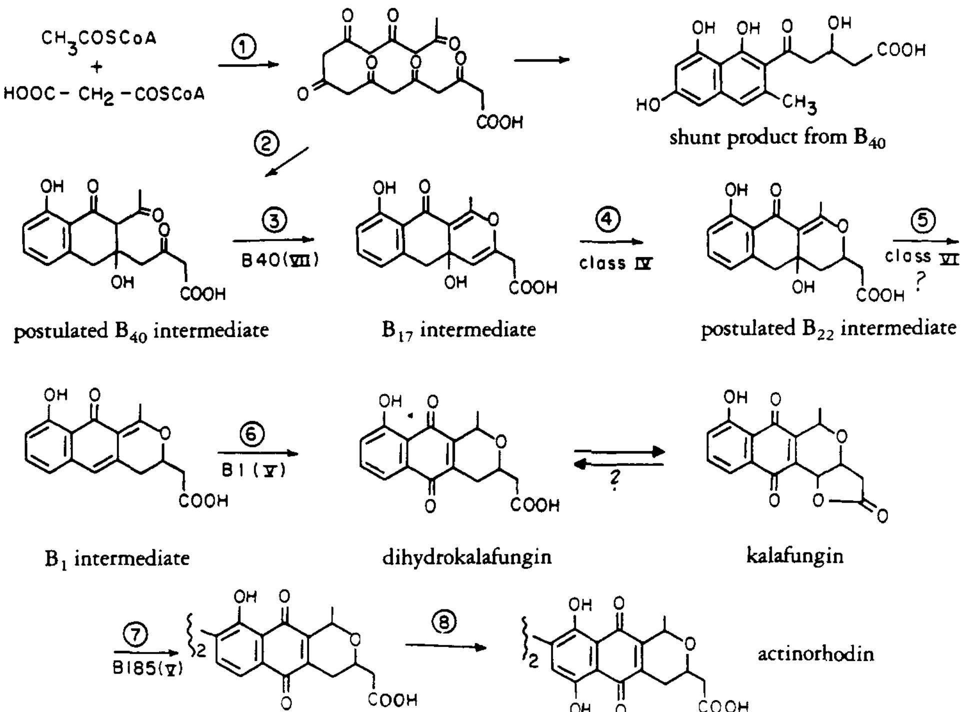

FIGURE 18. Hypothetical biosynthetic pathway to actinorhodin

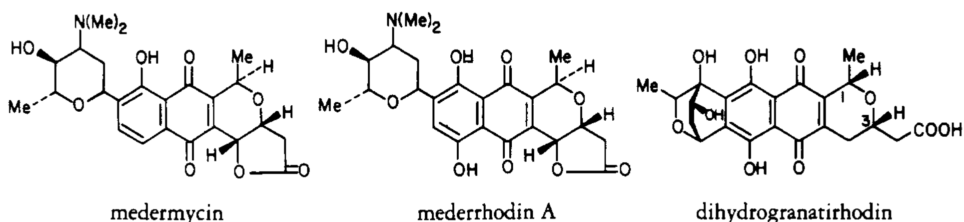

FIGURE 19. Structures of medermycin and the new hybrid antibiotics mederrhodin A and dihydrogranatirhodin

of actinorhodin (32). Transformation of a granaticin-nonproducing mutant of *Streptomyces violaceoruber* by plasmids containing several of the actinorhodin biosynthetic genes led, in some cases, to restoration of pigment production. Analysis of the pigment in this case showed that it was normal dihydrogranaticin, demonstrating that one of the actinorhodin biosynthetic genes could function in place of the corresponding granaticin pathway gene despite the fact that the enzyme normally operates on a substrate of opposite stereochemistry and different structure. A new compound was produced by a transformant of the *S. violaceoruber* wild type with the plasmid containing the entire actinorhodin biosynthesis gene cluster. The transformant was found to produce actinorhodin and a new compound, dihydrogranatirhodin (Figure 19) which has all of the structural features of dihydrogranaticin but the stereochemistry of one of the chiral centers is switched from that of granaticin to that of actinorhodin (32). These compounds are the first two examples of hybrid antibiotics produced by genetically engineered organisms which carry and express genetic information from two different parents. The structural modifications achieved here are very small, but they demonstrate a very important principle. Its applicability is now being explored in more complex systems in a number of laboratories, including our own.

The above examples, we hope, have demonstrated both the type of questions one asks in biosynthetic studies and the different approaches that are used in such investigations. The last example, hopefully, has also illustrated one way biosynthetic knowledge can be used to produce new natural product structures.

ACKNOWLEDGMENTS

We wish to acknowledge the enthusiastic and dedicated contributions of a large number of graduate students and postdoctoral fellows in this work. Their names are listed in the appropriate references. In addition, we have greatly enjoyed the extensive collaborations with several laboratories around the world, particularly those of Professors Hopwood in Norwich, Ōmura in Tokyo, and Zeeck in Göttingen. Finally, we appreciate the financial support of our work by the National Institutes of Health.

LITERATURE CITED

1. K. Kakinuma, N. Ikekawa, A. Nakagawa, and S. Ōmura, *J. Am. Chem. Soc.*, **101**, 3402 (1979).
2. U. Hornemann, J.P. Kehrer, C.S. Nunez, and R.L. Ranieri, *J. Am. Chem. Soc.*, **96**, 320 (1974).
3. K.L. Rinehart, J.M. Potgieter, W.Z. Jin, C.J. Pearce, and D.A. Wright, "Proc. Int. Conf. Trends in Antibiotics Research," Tokyo, 1982, pp. 171-184.
4. J.J. Kibby, I.A. McDonald, and R.W. Rickards, *J. Chem. Soc. Chem. Comm.*, 768 (1980).
5. M.G. Anderson, J.J. Kibby, R.W. Rickards, and J.M. Rothschild, *J. Chem. Soc. Chem. Comm.*, 1277 (1980).
6. O. Ghisalba, and J. Nüesch, *J. Antibiot.*, **34**, 64 (1981).
7. K. Hatano, S. Akiyama, M. Asai, and R.W. Rickards, *J. Antibiot.*, **35**, 1415 (1982).
8. S.W. Lee, Ph.D. dissertation, Purdue University, West Lafayette, Indiana 1983.
9. S. Horii and Y. Kameda, *J. Chem. Soc. Chem. Comm.* 747 (1972).
10. E. Truscheit, W. Frommer, B. Junge, L. Müller, D.D. Schmidt, and W. Wingender, *Angew. Chem. Int. Ed. Engl.*, **20**, 744 (1981).
11. U. Degwert, H. Pape, R.E. Herrold, J.M. Beale, P.J. Keller, J.P. Lee, and H.G. Floss, *J. Antibiot.* (submitted).

12. A. Bax, R. Freeman, and T.A. Frenkiel, *J. Am. Chem. Soc.*, **103**, 2102 (1981).
13. L. Müller, *J. Magn. Reson.*, **59**, 326 (1984).
14. J.M. Beale, P.J. Keller, C.E. Cottrell, and H.G. Floss, *J. Magn. Reson.* (submitted).
15. R.v. Hülst, H. Pape, B. Junge, L. Müller, J. Pfitzner, H. Schutt, manuscript in preparation.
16. K. Schröder and A. Zeeck, *Tetrahedron Lett.*, 4995 (1973).
17. K.L. Rinehart, M. Potgieter, D.L. Delaware, and H. Seto, *J. Am. Chem. Soc.*, **103**, 2099 (1981).
18. Y. Konda, M. Onda, K. Hinotozawa, S. Ōmura, and H.G. Floss, *J. Antibiot.*, **34**, 1222 (1981).
19. M. Ojika, H. Niwa, Y. Shizuri, and K. Yamada, *J.Chem. Soc. Chem. Comm.*, 628 (1982); and references therein.
20. P. Welzel, F.J. Witteler, D. Müller, and W. Reimer, *Angew. Chem.*, **93**, 130 (1981).
21. H. Nakano, M. Yoshida, K. Shirahata, S. Ishii, Y. Arai, M. Morimoto, and F. Tomita, *J. Antibiot.*, **35**, 760 (1982).
22. A. Nakagawa, T.S. Wu, P.J. Keller, J.P. Lee, S. Ōmura, H.G. Floss, *J. Chem. Soc. Chem. Comm.*, 519 (1985).
23. J.M. Beale, J.P. Lee, A. Nakagawa, S. Ōmura, and H.G. Floss, *J. Am. Chem. Soc.*, **108**, 331 (1986).
24. B. Sedgwick, J.W. Cornforth, S.J. French, R.F. Gray, E. Kelstrup, and P. Willardsen, *Eur. J. Biochem.*, **75**, 481 (1977).
25. S. Huang, J.M. Beale, P.J. Keller, and H.G. Floss, *J. Am. Chem. Soc.*, **108**, 1100 (1986).
26. C.P. Gorst-Allman, B.A.M. Rudd, C.j. Chang, and H.G. Floss, *J. Org. Chem.*, **46**, 455 (1981).
27. C.E. Snipes, C.j. Chang, and H.G. Floss, *J. Am. Chem. Soc.*, **101**, 101 (1979).
28. X.G. He, C.C. Chang, C.j. Chang, J.C. Vederas, A.G. McInnes, J.A. Walter, and H.G. Floss, *Z. Naturforsch.*, **41C**, 215 (1986).
29. B.A.M. Rudd and D.A. Hopwood, *J. Gen. Microbiol*, **114**, 35 (1979).
30. H.G. Floss, S.P. Cole, X.G. He, B.A.M. Rudd, J. Duncan, I. Fujii, C.j. Chang, and P.J. Keller, in: "Regulation of Secondary Metabolite Formation." Ed. by H. Kleinkauf *et al.* Proceeding of the 16th Workshop Conference Hoechst, Cologne, May 1985, pp. 283-304.
31. F. Malpartida and D.A. Hopwood, *Nature*, **309**, 462 (1984).
32. D.A. Hopwood, F. Malpartida, H.M. Kieser, H. Ikeda, J. Duncan, I. Fujii, B.A.M. Rudd, H.G. Floss, and S. Ōmura, *Nature*, **314**, 642 (1985).
33. P.M. Jordan, J.B. Spencer, and D.L. Corina, *J. Chem. Soc., Chem. Commun.*, 911 (1986).