Biosynthesis of the Kinamycins

Steven J. Gould

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331

Received February 4, 1997 (Revised Manuscript Received June 16, 1997)

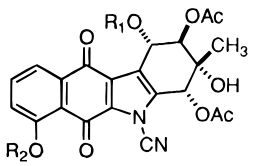
Contents

Steven J. Gould was born in New York City, NY, in 1946. He received his B.S. from the University of California at Los Angeles in 1966 and his Ph.D. from the Massachusetts Institute of Technology in 1970, having studied under the direction of Professor George Büchi. He then spent two years as a postdoctoral associate at the Eidgenossische Technische Hochschule with Professor Duilio Arigoni; during the first year he was a Fellow of the American–Swiss Foundation for Scientific Exchange. In 1972, he joined the Syva Research Institute in Palo Alto, CA, and in 1974 he accepted a position as Assistant Professor of Pharmacognosy at the University of Connecticut. He was promoted to Associate Professor in 1980, and in 1982, he moved to the Department of Chemistry at Oregon State University where, until recently, he was Professor of Chemistry and Biochemistry. In 1997, he accepted the position of Executive Director of Natural Product Drug Discovery at Merck Research Laboratories in Rahway, NJ. He has held a Research Career Development Award from the NIH, an American Cancer Society Scholar Award, and a fellowship from the Fulbright Commission. His research interests have focused on the biosynthesis of antibiotics, applying structural, biochemical, and molecular genetics techniques to a variety of polyketides, nucleosides, and nonribosomal peptides, and other amino acid-derived natural products.

I. Introduction

The biosynthesis of the kinamycin antibiotics has been studied primarily at the structural level, but recently at the biochemical and molecular genetics levels, as well. Although the kinamycins have been known in the literature since 1970,[1−6] an incorrect identification of the fundamental skeleton formed the basis of all work until the correct skeleton was determined in 1994.[7,8] As a result, a thorough review of kinamycin biosynthesis requires inclusion of an historical view of assumed biogenetic connections to other metabolites. These later proved to be nearly correct, albeit in a manner not originally anticipated. Thus, while the kinamycins were believed for 23 years to be *N*-cyanobenzo[*b*]carbazoles (e.g, **1** for kinamycin D), they are in fact diazo-substituted benzo[*b*]fluorenes (e.g., **2** for kinamycin D, Table 1), although the fundamental derivation from a benzo[*a*]anthraquinone was correctly established in 1987.[9] Many of the key recent findings have made use of synthesized putative advanced biosynthetic intermediates, and a review of efforts toward the synthesis of the benzo[*b*]carbazole and benzo[*b*]fluorene skeletons by various laboratories will be included. Some aspects of kinamycin biosynthesis have been reviewed.[10] The period covered by this review is from 1970 to 1996.

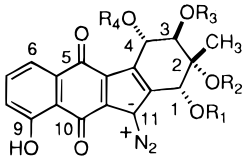
Four kinamycins were originally isolated from *Streptomyces murayamaensis* sp. nov. Hata and Ohtani (**2**−**5**, correct structures shown).[3] These bright golden-yellow compounds are strongly active against Gram-positive bacteria but less so against Gram-negative organisms. Kinamycin C showed weak antitumor activity. The structures were characterized by Ōmura *et al.* as possessing a benzo[*b*]-tetrahydrocarbazole skeleton with an *N*-cyano moiety, both of these features being extremely rare. These were determined in part by chemical and spectroscopic means. The structure and absolute stereochemistry of kinamycin C was completed by an X-ray crystallographic analysis of the *p*-bromobenzoate **20**.[5]



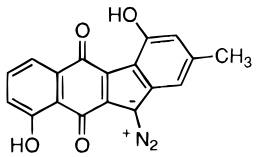
1, $R_1 = R_2 = H$
20, $R_1 = H$, $R_2 = p$-BrPhCO

S0009-2665(96)00021-0 CCC: $28.00

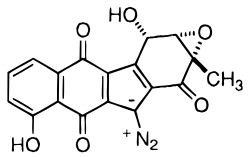
Table 1



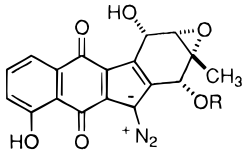
			R_1	R_2	R_3	R_4
3	Kinamycin	A	H	Ac	Ac	Ac
4		B	H	Ac	H	H
5		C	Ac	H	Ac	Ac
2		D	Ac	H	Ac	H
6		E	Ac	H	H	H
7		F	H	H	H	H
8		G	Ac	CO*i*Pr	Ac	Ac
9		H	CO*i*Pr	H	Ac	Ac
10		I	CO*i*Pr	H	CO*i*Pr	Ac
11		J	Ac	Ac	Ac	Ac
12	FL-120 A		Ac	CO*i*Pr	Ac	H
13	FL-120 C		CO*i*Pr	H	Ac	H
14	FL-120 C'		COEt	H	Ac	H
15	FL-120 D'		CO*i*Pr	H	H	H



16, Prekinamycin



17, Ketoanhydrokinamycin



18, FL-120B, R = Ac
19, FL-120B', R = CO*i*Pr

While the producing organism was assigned to the genus *Streptomyces*, it seemed to resemble *Nocardia* because of the fragmentation of the mycelium. Both the original strain and the patent strain (ATCC 21414) at first failed to produce aerial mycelium and appeared as firm, dark chocolate-brown colonies on standard agar media. However, a lengthy incubation on an oatmeal-based agar led to the formation of isolated tufts of white aerial mycelium and to spore formation. Subculturing led to a strain that formed these structures reproducibly, and these are produced more abundantly on ISP4 agar. The morphology, chemical composition, and physiological properties then led to recognition of a closer connection to *Streptoverticillium*.[11]

Until 1989, *S. murayamaensis* was the only organism known to produce kinamycins. However, in 1989[12] and 1992,[13] two other kinamycin-producing microorganisms were reported. One organism is of the genus *Saccharothrix* (produces **9** and **10**), and the other is an unidentified actinomycete (produces **11**, antibiotic A83016A). In 1994, *Streptomyces chattanoogensis* subsp. *taitungensis* was reported to produce kinamycin D and six new kinamycins, **12**−**15** (FL-120A, -120C, -120C′, and -120D′, respectively) and **18** and **19** (FL-120B and -120B′).[14,15]

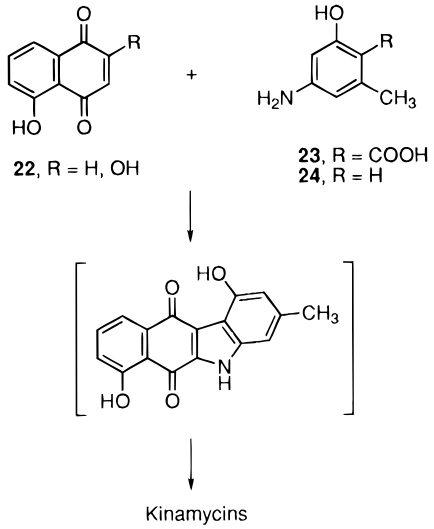
II. Biosynthetic Studies

A. Initial Studies

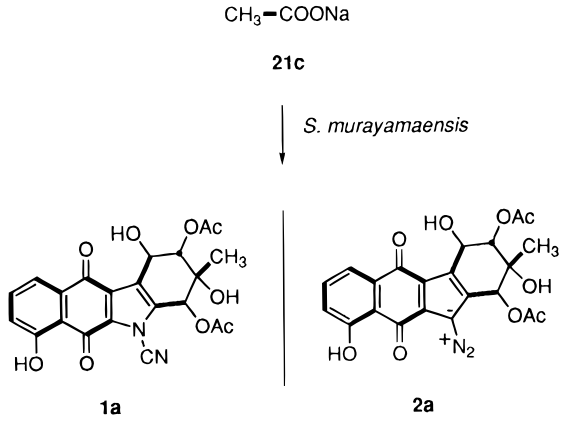
The biosynthesis of the kinamycins was first examined by Ōmura,[16] who fed sodium [1-^{13}C]acetate (**21a**) and sodium [2-^{13}C]acetate (**21b**) to *S. murayamaensis*. The derived kinamycins were analyzed by IR spectroscopy, a novel approach to biosynthetic studies. An isotope-induced shift of the putative cyanamide ^{13}C−N triple bond stretching frequency from 2155 to 2139 cm^{-1} was noted only for the former, and it was suggested that the cyanamide carbon was derived from C-1 of acetate.

Sato *et al.*[17] next explored the biosynthesis of these compounds on the basis of a retrobiogenetic analysis that anticipated that the benzo[*b*]carbazole skeleton was derived from coupling a hydroxylated naphthoquinone, **22**, and either 3-amino-5-hydroxytoluic acid, **23**, or the product of its decarboxylation, **24**, as shown in Scheme 1.[18,19] There was precedent for either

Scheme 1

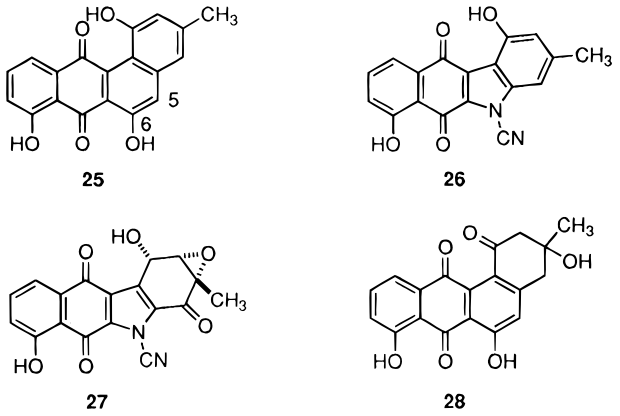


precursor to be derived by either a polyketide[20−23] or shikimate-based pathway,[24−26] or a combination of both.[27,28] Sodium [1,2-$^{13}C_2$]acetate (**21c**) was fed to *S. murayamaensis* grown in a medium that allowed high production of kinamycin D in the presence of such nonphysiological concentrations of acetate. ^{13}C NMR analysis of the derived metabolite, **1a** (**2a**), clearly showed that the entire skeleton was of polyketide origin. However, the cyanamide resonance could not be found, so its genesis remained unknown; the region of 110−120 ppm—typical for cyanamides—contained no resonances. In this particular fermentation, approximately equal amounts of labeled kinamycin D and kinamycin C (**5a**) were formed, which is atypical of this organism in the medium used.



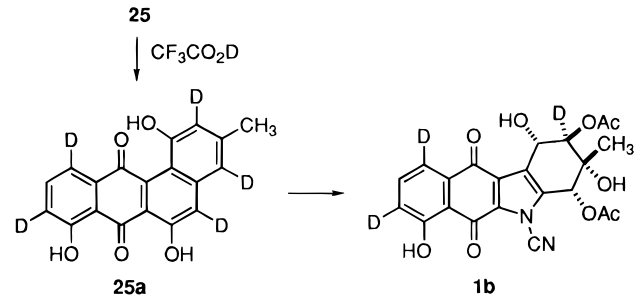
B. New Metabolites, ^{15}N Labeling, and the Cyanamide Carbon

Three avenues of inquiry, carried on simultaneously, ultimately led to clarification of the pathway leading to the nominal benzo[*b*]carbazole skeleton and the cyanamide carbon. A variety of deuterated hydroxynaphthalenes and hydroxynaphthoquinones were synthesized and fed to *S. murayamaensis*; however, ^{2}H NMR analysis indicated that none had been incorporated.[9] *S. murayamaensis* produces many colored metabolites when grown in a variety of media. A number of these were isolated and characterized (**6**, **7**, and **25**−**27**), and structure **25** gave insight into the failure of the naphthalenes to label kinamycin D. It was recognized that excision



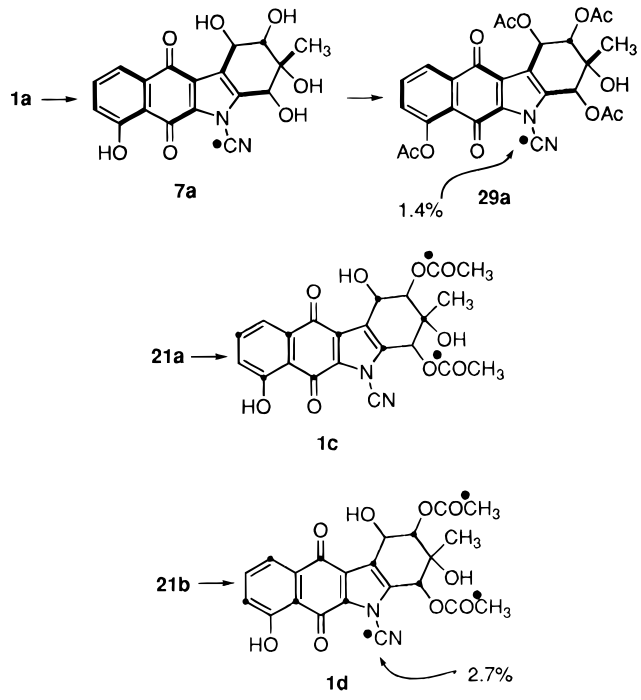
of the two-carbon unit C-5/C-6 of the angular ring, followed by insertion of nitrogen would yield the kinamycin skeleton with the observed acetate labeling pattern. A deuterated sample, **25a**, prepared by acid-catalyzed exchange, was fed to *S. murayamaensis* and ^{2}H NMR analysis confirmed that **1b** had been produced.[9,29] Compound **25** had previously been obtained from acid-catalyzed dehydration of rabelomycin, **28**, from *S. olivaceus*,[30] and the former had been named dehydrorabelomycin. It was now shown to be a precursor to the kinamycins.[9]

The third avenue was incorporation of a general nitrogen source enriched in ^{15}N as a way to locate the nominal cyanamide carbon. A synthetic liquid medium was developed containing ammonium sulfate as the sole nitrogen source. [$^{15}N_2$]Kinamycin D was obtained by using $(^{15}NH_4)_2SO_4$,[31] and the ^{15}N NMR spectrum showed two doublets (J = 3.4 Hz), in addition to the signal from the external reference,



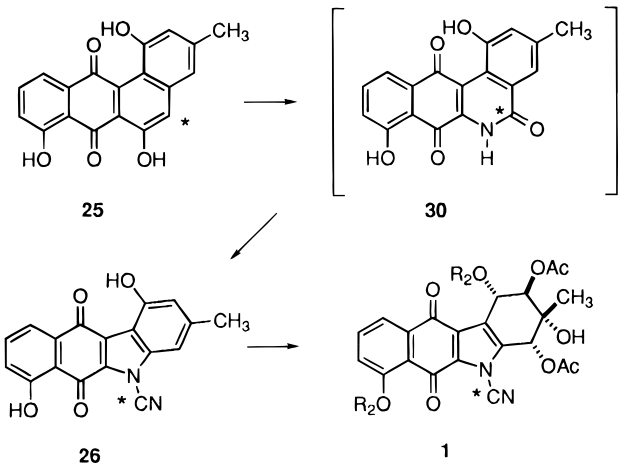
$H^{15}NO_3$. The corresponding ^{13}C NMR spectrum surprisingly now contained a doublet of doublets at δ 78.5 (J = 21.2 and 5.4 Hz). Reexamination of ^{13}C NMR spectra of natural abundance kinamycin samples showed a very small singlet at this chemical shift, almost overlapped by the solvent resonance ($CDCl_3$).

With the nominal cyanamide resonance now recognized, all typical one-carbon precursors were tested. None labeled this carbon exclusively, and only [3-^{13}C]serine and [2-^{13}C]glycine afforded any labeling. These had been metabolized to labeled acetate **21a** and **21c**, respectively. The kinamycin D, **1a**, originally obtained by feeding **21c** directly was carefully hydrolyzed, and the desacetylkinamycin, **7a**, reacetylated to give the tetraacetate **29a** in order to provide resonances at natural abundance for reference. The resonance at δ 78.5 was now found to be 1.4% enriched, similar to the average enrichment for the ring carbons.[31] Separate feedings of **21a** and **21b** now provided **1c** and **1d**, respectively, which clearly demonstrated the one-carbon unit had originated from C-2 of acetate. This data, combined with the



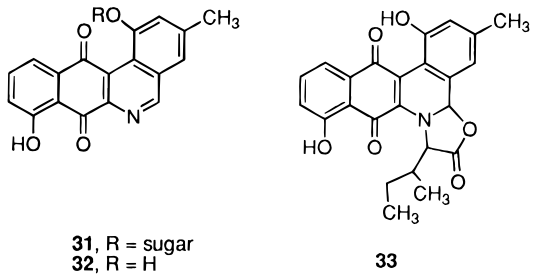
almost simultaneous characterization of a purple *S. murayamaensis* metabolite as **26** (given the name prekinamycin),[29] led to the hypothesis that the pathway from dehydrorabelomycin proceeded via oxidative ring cleavage, nitrogen insertion, and ring contraction/rearrangement to **26** as shown in Scheme

Scheme 2



2, with benzo[*b*]phenanthridine(s) (e.g., **30**) as key intermediate(s).[31]

Although no naturally occurring benzo[*b*]phenanthridines had as yet been observed, the first members of this new skeletal class appeared in the literature almost immediately. Phenanthroviridin (**31**) and its aglycon **32** were reported by a group at Ciba-Geigy,[32] and the jadomycins (e.g., jadomycin A, **33**) were reported by a Canadian group.[33,34] Both **32** and the

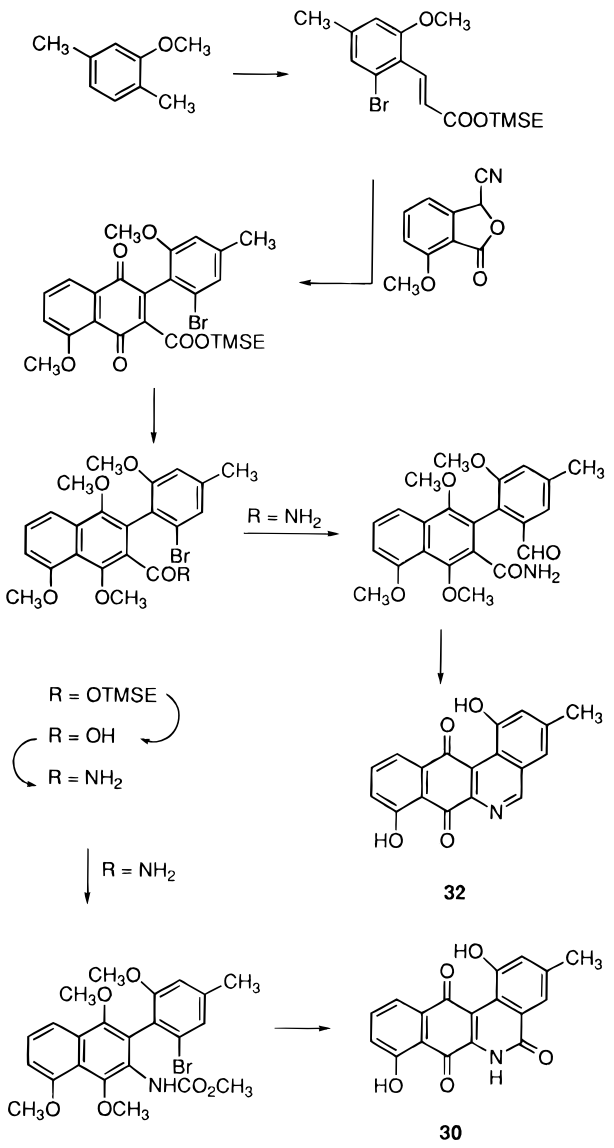


analogous pyridone **30** were subsequently synthesized (Scheme 3).[35,36] Deuterated samples were prepared and fed to *S. murayamaensis*, but no incorporation was observed. No clear conclusions were drawn, however, because both compounds were extremely insoluble and the samples fed were recovered essentially quantitatively. Phenanthroviridin aglycon was subsequently identified in extracts of *S. murayamaensis* strain MC2, a mutant blocked in kinamycin biosynthesis.[37]

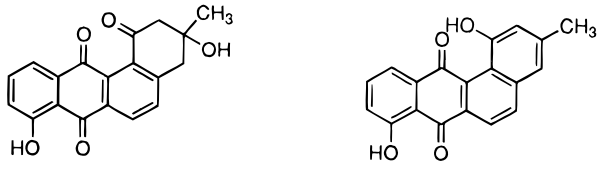
C. Biosynthesis of Benzo[*a*]anthraquinones (Angucyclinones)

The first naturally occurring benza[*a*]anthraquinones characterized were tetrangomycin (**34**) and tetrangulol (**35**) produced by *S. rimosus* NRRL 3016,[38] and rabelomycin (**28**) was reported from *S. olivaceus* a few years later, in 1970.[30] This group remained a minor subset of polycyclic aromatic polyketides for a number of years, but now constitutes the largest known group of such decaketide metabolites. As a group, they have been called angucyclinones[39] and isotetracenomycins;[40] the first of these has become the one commonly used, and these were reviewed in 1992.[10] Incorporation of labeled acetates has been reported for vineomycin B[41] and for urdamycins A and B,[42] as well as for dehydrorabelomycin. In each

Scheme 3



case, the same labeling pattern was observed. Although two different foldings of the decaketide in-

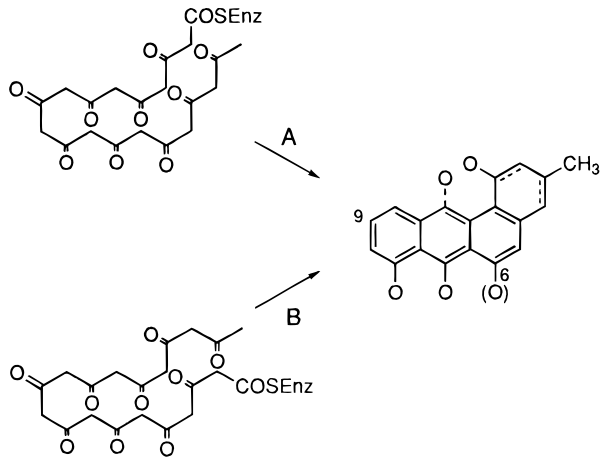


termediate would theoretically be possible, only the one shown in Scheme 4, route A, seems to be utilized. In addition, in all cases the C-9 oxygen has been lost, and in many cases the C-6 has, as well.

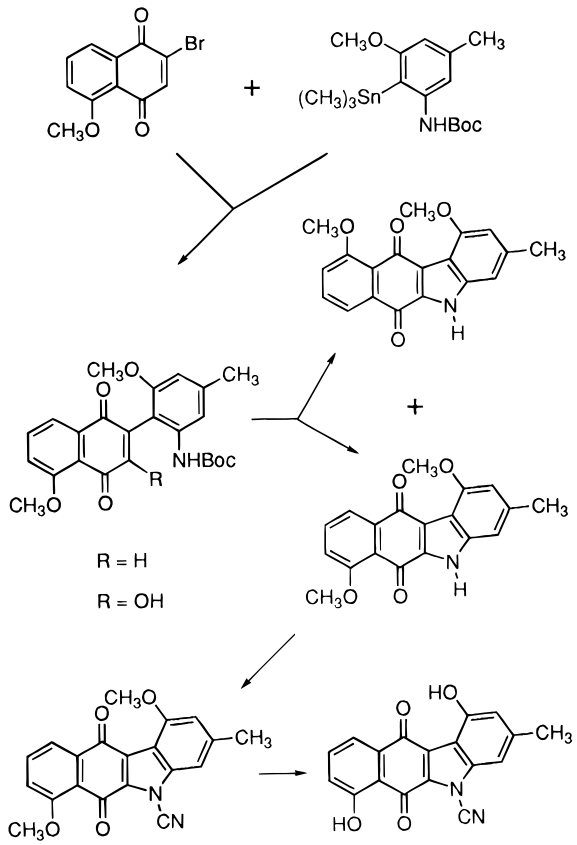
D. Synthesis of the Putative Prekinamycin and Revised Structures for the Kinamycins

Numerous groups[43−47] worked toward the synthesis of the *N*-cyanobenzo[*b*]carbazole **26** that was believed to be prekinamycin.[43,44,47] The IR (2237−2245 cm^{-1}) and ^{13}C NMR (δ 105−108) data for a set of *N*-cyanoindoles prepared by Dmitrienko *et al.*[43] agreed poorly with data from the kinamycins (2119−2170

Scheme 4



Scheme 5

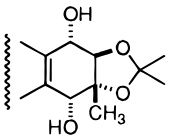


26

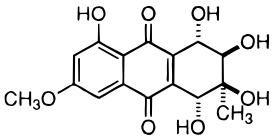
cm^{-1} and δ ~78, respectively), as did intermediates in the Echavarren synthesis (δ 115−123).[48] These raised serious concerns about the kinamycin structure, and this culminated in Echavarren's synthesis (Scheme 5),[48] which provided **26** with spectroscopic data that did not match that of the natural product.

^{1}H NMR spectroscopic data had been used extensively by Ōmura *et al.*[4] to identify the substitution patterns of the naphthoquinone and of the highly oxygenated D-ring of the kinamycins. However, these two spin systems were separated by too many quaternary carbons to be correlated with each other, even with the long-range heteronuclear spin coupling NMR experiments developed in recent years. The unusual cyanamide moiety had been assigned from the IR absorbance and from detection of ammonia upon subjecting the kinamycins to hydrolytic conditions.[4] The X-ray diffraction study[5] of **20** appeared to confirm these features. Interestingly, a second incorrect X-ray crystallographic analysis of a kinamycin was reported recently.[15] Although the nominal cyanamide carbon ^{13}C NMR resonance was subsequently detected ~30 ppm up field from those of simple cyanamides, the unusual chemical shift was attributed to possible electronic effects of the indoloquinone.

The C-2/C-3 relative stereochemistry of the kinamycins was reported to be *trans*, and desacetylkinamycin was claimed to form the acetonide **36**.[4] A nuclear Overhauser effect difference spectrum NMR experiment (NOEDS) on kinamycin A83016 (kinamycin I) supported this.[13] However, altersolanol A, **37**, has been shown to have the opposite stereochemistry.[49−51]

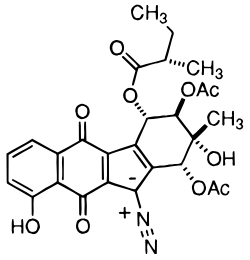


36



37

A new crystalline derivative of kinamycin D containing a stereogenic center was prepared in order to resolve these issues. The (+)-α-methylbutyrate **38** was prepared using the acid anhydride and $ZnCl_2$ catalysis, and a single crystal, obtained by diffusion of water into a DMF solution, was analyzed by X-ray diffraction.[7] The data was solved for the diazo-substituted benzo[*b*]fluorene, and for a benzo[*b*]carbazole substituted at nitrogen with either a nitrile or isonitrile moiety, and this clearly identified the diazo structure as the correct one. Prekinamycin was therefore assigned structure **16**.



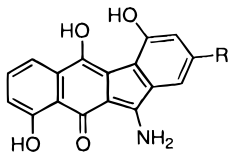
38

E. Benzo[*b*]fluorenes in Kinamycin Biosynthesis

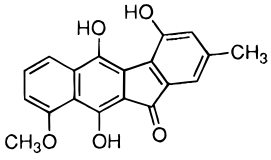
Prior to 1993, naturally occurring benzo[*b*]fluorenes were unknown. Once again, work on kinamycin biosynthesis seemed to catalyze discovery of such compounds. A set of *S. murayamaensis* mutants blocked in kinamycin biosynthesis had been generated by random mutagenesis.[52] A number of these accumulated additional colored metabolites, and each of those that were then structurally characterized was subsequently detected at low levels and/or at specific time points in fermentations of the wild strain when grown in selected media.

The first three natural products of the class were published within months of each other. Shin-ya *et*

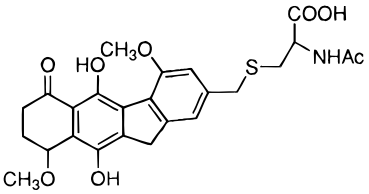
al.[53] reported stealthins A and B, **39** and **40**, respectively, as free-radical scavengers produced by *S. viridochromogenes*. Cone *et al.*[54] reported kinafluorenone, **41**, the major colored metabolite of *S. murayamaensis* mutant strain MC2. The quinone form of kinafluorenone was detected after autooxidation of extracts and recognized as such by its conversion back to the hydroquinone in the presence of sodium dithionite.[52] Until the kinamycins were recognized to be diazobenzo[*b*]fluorenes, kinafluorenone was thought to be a "shunt" metabolite derived from an intermediate before nitrogen insertion. However, with the correct kinamycin structure it was recognized that kinafluorenone may be removed from the main pathway by only one step, *O*-methylation. These were soon followed by the report of cysfluoretin (**42**), an inhibitor of glutathione *S*-transferase, produced by an unidentified *Streptomyces* species.[55]



39, R = CH_2OH
40, R = CHO



41

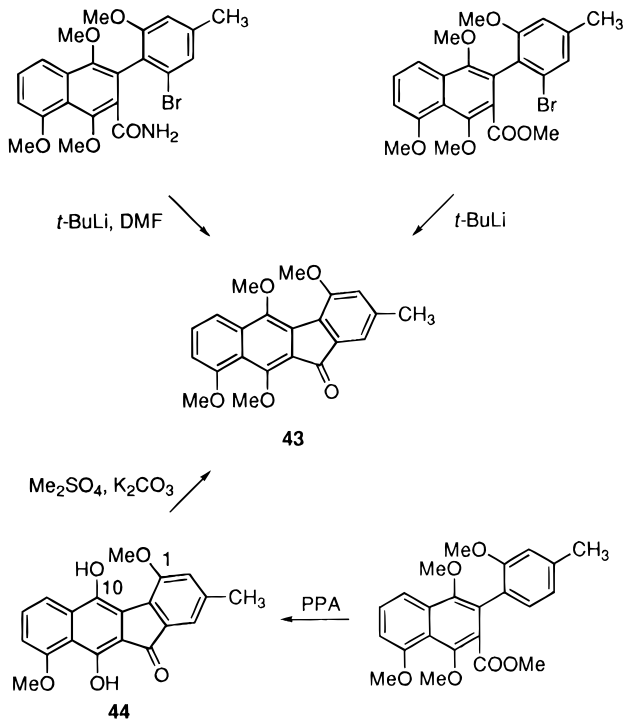


42

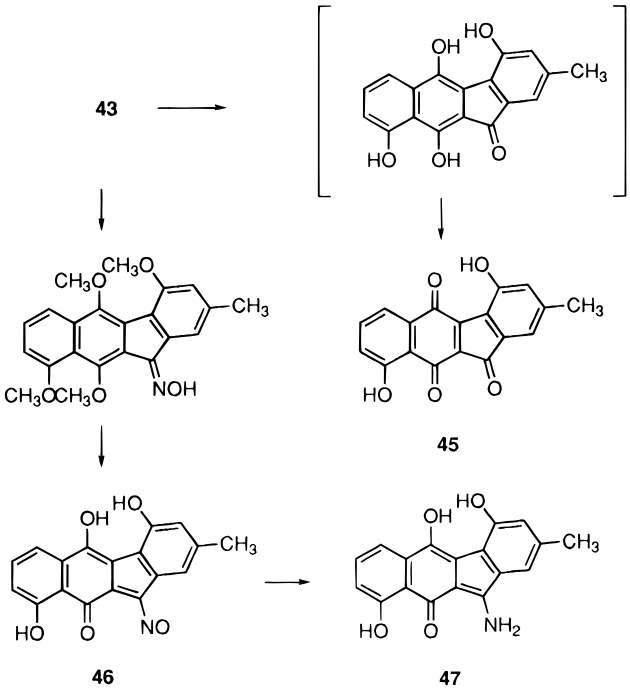
Further advances in understanding kinamycin biosynthesis have relied on the combination of synthesis of potential advanced intermediates, photodiode array-detected HPLC, and the set of kinamycin-negative *S. murayamaensis* mutants. The mutants had been selected from survivors of either UV- or chemical mutagenesis. Selection was based upon lack of antibiotic activity using agar-disk assays against *B. subtilis* ATCC 6633, the lack of apparent morphological abnormalities, and—in some cases—the presence of unusual colors of the colonies growing on agar. Such candidates were then analyzed by TLC of organic extracts of agar plugs and roughly classified by the sets of colored metabolites each produced on a number of agar media. Definitive classifications were made when photodiode array-detected HPLC became available. Although most of the colored metabolites were still of unknown structure, apparent families of metabolites were recognizable from their UV−vis spectra.

Ironically, the identification of these metabolites was facilitated by the formation of a minor side product during the synthesis of the phenanthridine previously believed to be involved in kinamycin biosynthesis. Tri-*O*-methylkinafluorenone (**43**) was the first benzo[*b*]fluorene synthesized[36] when it was obtained as a minor side product in the synthesis of phenanthroviridin aglycon (Scheme 6). Subsequently, it was obtained in high yield when DMF was left out of the reaction.[56] Variations of this approach have

Scheme 6



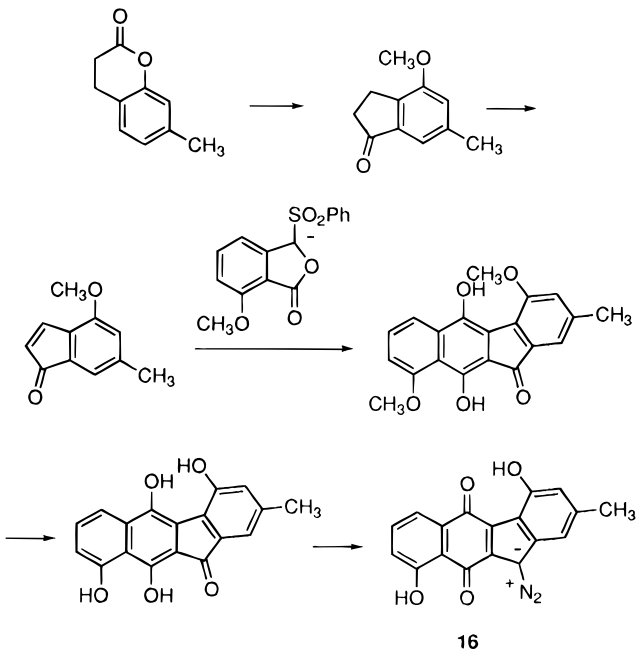
Scheme 7



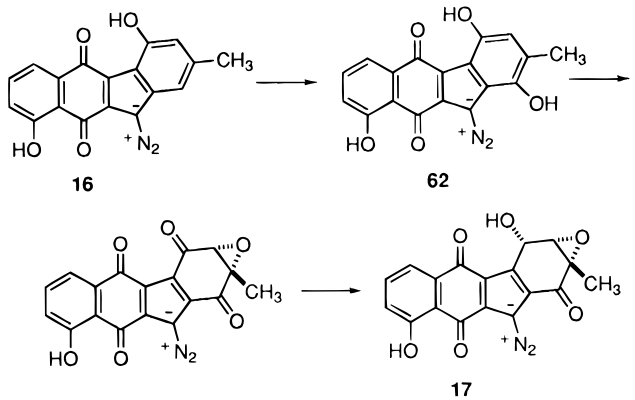
led to a shorter, higher yielding route to both **43** and to derivative **44** with the unprotected hydroquinone.[57] The remarkable ease with which the hydroquinone undergoes deprotection was also observed in earlier studies on the synthesis of the benzo[*a*]anthraquinone tetrangulol, **35**.[58] This may be due to distortion of the ring system from planarity as a result of the steric crowding between the oxygen substituents at C-1 and C-10.

The unprotected benzo[*b*]fluorenone **45**, and its nitroso and amino analogs, **46** and **47**, respectively, have been synthesized as shown in Scheme 7. With authentic samples available, *S. murayamaensis* was

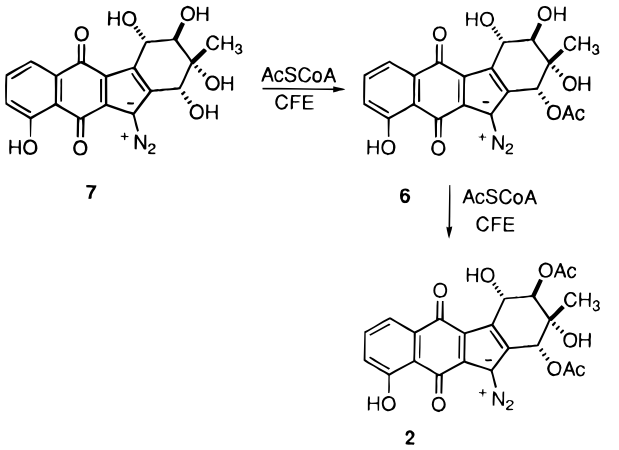
Scheme 8



Scheme 9



Scheme 10



examined for their production. Although **45** was not observed in extracts made from fermentation broths worked up in standard fashion, it was later detected between 12 and 24 h after inoculation of a production broth, but it was no longer detectable by 36 h.[59] The aminobenzo[*b*]fluorene **47** was detectable in initial extracts but it was not obtainable from extracts worked up in standard fashion. A modified, gentler work up using Sephadex LH-20 chromatography was successful.[57] All three compounds have been prepared with deuterium labels and fed to *S. murayamaensis*. Both the ketone—kinobscurinone[59]—and the amine—stealthin C[57]—were specifically incorporated into kinamycin D.

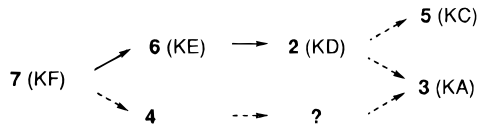
Hauser's group[60] has reported the first synthesis of prekinamycin (**16**), shown in Scheme 8. This provides an alternative route to dimethylkinobscurinone (**44**). Surprisingly, once again, the IR and NMR data did not match the metabolite previously assigned this structure, and its analysis by photodiode array-detected HPLC showed it to have both a different retention time and a different UV—vis spectrum. However, the latter analysis did identify the correct *S. murayamaensis* metabolite for this structure.[61] A deuterated sample was synthesized and fed to, but was not incorporated into, kinamycin D.[62] However, prekinamycin has proven to be even less soluble than the benzo[*b*]phenanthridines described above. Other approaches to the synthesis of fluorenes and benzo[*b*]fluorenes have recently been reported.[63,64]

F. The Endgame: Elaboration of the Kinamycin D-Ring

One of the first *S. murayamaensis* cometabolites characterized—ketoanhydrokinamycin (**17**)[29]—contains an epoxyquinol moiety. This moiety, and the analogous epoxyquinone, are encountered in numerous microbial metabolites. Also, an epoxyquinone is generated from dihydrovitamin K during its involvement in the carboxylation of glutamate residues of prothrombin in mammals.[65–67] The biosynthesis of antibiotic LL-C10037α has been shown to proceed via oxidation of a phenol to a hydroquinone, followed by epoxidation directly to an epoxyquinone, and then reduction to the epoxyquinol.[68–70] On the basis of this example, an analogous set of reactions is expected for the conversion of prekinamycin to ketoanhydrokinamycin, as shown in Scheme 9.

The early biosynthetic studies had identified kinamycin F (**7**), as a metabolite of *S. murayamaensis*,[29] and it was apparent that it was the branch point to the variously acetylated kinamycins and, more recently, those modified with other short chain fatty acids (Table 1). Kinamycin F is readily prepared by basic hydrolysis of acetylated kinamycins and has been used to examine the acetyltransferases of *S. murayamaensis*. So far, only the details leading to kinamycin D have come to light. Cell-free activity was readily detected for the conversion of **7** to kinamycin D, and proof of kinamycin E (**6**) as an intermediate was obtained in the process (Scheme 10).[71] The latter was first obtained as the minor monoacetate from brief treatment of **2** with base at ice-bath temperature.[31] A partial purification of this dual acetyltransferase activity (kinamycin acetyltransferase I, KAT I) was obtained, and an apparently large (M_R >669 kDa), membrane-associated multifunctional enzyme was described. It was specific for the formation of kinamycin D. The membrane association, multifunctional nature, and large apparent size of KAT I are unusual features relative to other antibiotic acetyltransferases.[72–79]

Scheme 11

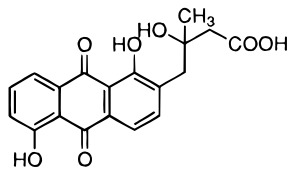


KAT I accounts for two of the five D-ring acetylation patterns observed in naturally occurring kinamycins. The minimum pathway which accounts for all reported acetylated kinamycins, shown in Scheme 11,[71] would require at least two additional KATs.

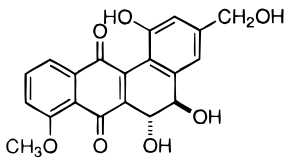
III. Molecular Genetics of Angucyclinone and Kinamycin Biosynthesis

As with numerous other aromatic polyketide pathways,[80] the polyketide synthase (PKS) genes for the kinamycin pathway, and for four other angucyclinone pathways have been detected using a fragment of *actI*,[81,82] the β-ketoacylsynthase gene from the actinorhodin pathway of *S. coelicolor* A2(3), as a probe. Vining[83,84] and Decker[85] cloned the PKS genes for the jadomycin and urdamycin pathways, respectively. Although an insufficient portion of the total gene cluster was cloned to afford metabolite production, in each of these cases gene disruption experiments demonstrated that the correct genes had been cloned.

By cloning larger fragments of genomic DNA from *S. rimosus* NRRL 3016 into *S. lividans* TK24, heterologous production of tetrangomycin (**34**), tetrangulol (**35**), and of fridamycin E (**48**) was obtained.[86] Using the same approach, sufficient DNA from *S.* strain WP 4669 was cloned for heterologous production[86] of antibiotic PD 116740 (**49**),[87] which is derived from tetrangulol.[88,89]

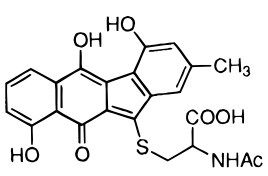


48



49

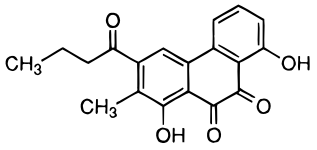
This latter protocol was also used to clone DNA from *S. murayamaensis*, although in this case *S. lividans* ZX7,[90,91] was used, a strain that appears not to be prone to plasmid homologous recombination. Although the complete kinamycin pathway was not captured on a single cosmid insert, sufficient DNA was cloned for heterologous production[92] of intermediates as far along as stealthin C.[57] In addition to this metabolite, dehydrorabelomycin and two shunt metabolites, kinafluorenone[54] and seongomycin (**50**),[93] were produced. The latter was the major colored metabolite of the four transformants that produced kinamycin-related metabolites.



50

IV. Other Metabolites of S. murayamaensis

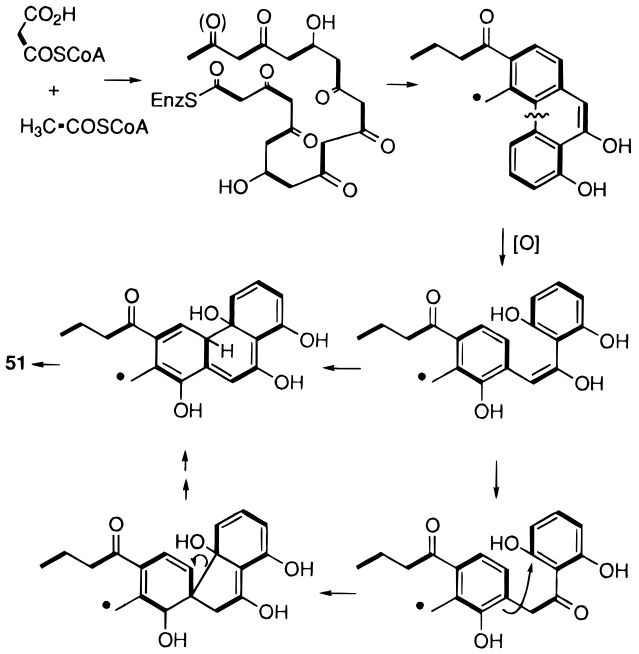
S. murayamaensis has proven to be a rich source of secondary metabolites from a variety of biosynthetic pathways. A second, and completely independent aromatic decaketide, murayaquinone (**51**) is also produced in substantial quantity under standard conditions.[94] Its biosynthesis involves a surprising



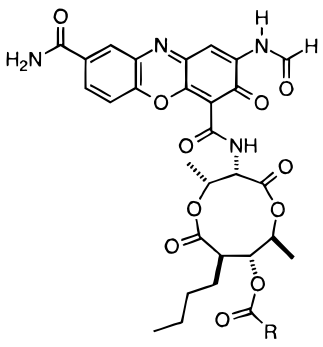
51

rearrangement, as shown in Scheme 12.[95]

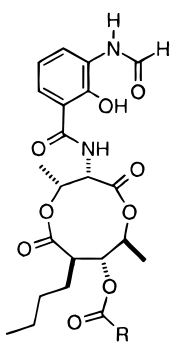
Scheme 12



A family of metabolites, the notantimycins, **52**−**54**, was discovered from extracts of *S. murayamaensis* mutant MC2,[96] and their biosynthesis includes C_6 and C_8 fatty acids.[97] The structural relationship to the antimycins (e.g., **55**−**57**) is clear, and the latter were detected from *S. murayamaensis* mutant MC2, as well as an antimycin-overproducer, mutant MC13.[98] The notantimycins can be viewed as antimycins that

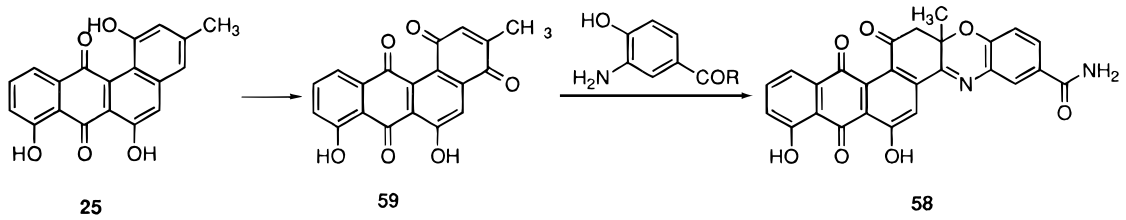


52, R = *sec*-Bu
53, R = *i*-Bu
54, R = *i*-Pr



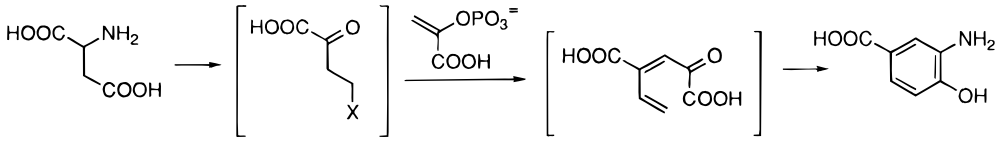
55, R = *sec*-Bu
56, R = *i*-Bu
57, R = *i*-Pr

Scheme 13



25 59 58

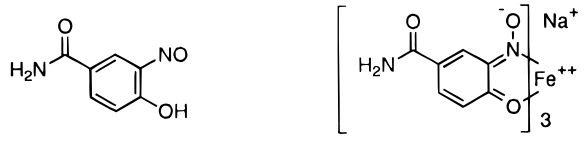
Scheme 14



have condensed with a 3-amino-4-hydroxybenzoic acid (3,4-AHBA) derivative. Both of these families were subsequently detected at low concentrations in extracts of the wild strain.

Murayaanthraquinone (**58**)[99] is another minor *S. murayamaensis* metabolite with a phenoxazone moiety derived in part from 3,4-AHBA. In this case, coupling has apparently occurred with **59** (PD 116744[100,101]) presumably derived from dehydrorabelomycin, **25**, as shown in Scheme 13.

Lastly, 3,4-AHBA also accumulates in fermentations of *S. murayamaensis* as the nitrosophenol **60** and its ferrous chelate **61**.[102] The biosynthesis of 3,4-



60 61

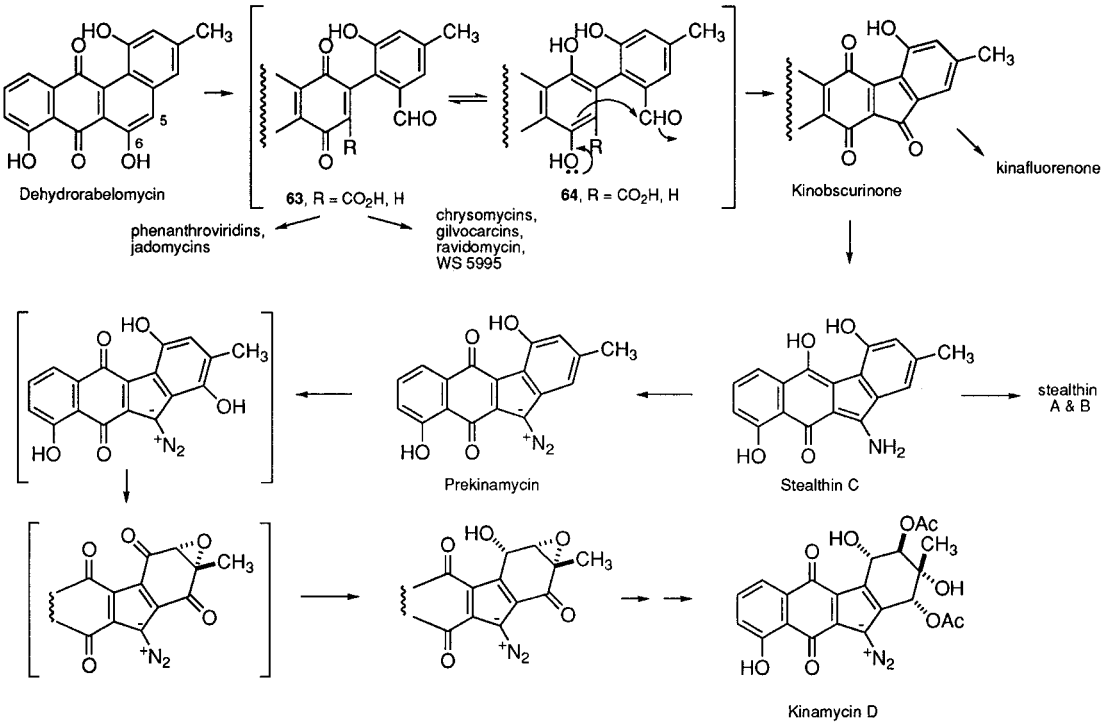
AHBA has recently shown it to be the first aminobenzoic acid isomer to be derived from a non-shikimic acid pathway. It is derived from a four-carbon unit from the citric acid cycle and a three-carbon unit from glycolysis (Scheme 14).[103] 3,4-AHBA has also been shown to be the precursor to the C_7N moiety found in manumycin and in asukamycin.[104]

V. Future Directions

Numerous significant mechanistic questions remain to be answered. Current efforts continue on the synthesis of additional putative intermediates and on the cloning and analysis of the kinamycin genes. Additional biochemical studies are likely to wait for enzymes made available from expression of cloned genes.

It appears that the same novel dioxygenase epoxidizing chemistry found in the LL-C10037α and MM14201 biosynthetic pathways and in the dihydrovitamin K epoxidase/carboxylase reaction is present in kinamycin biosynthesis (Scheme 9). The synthesis of the hydroquinone **62** is in progress in order to test its intermediacy and to provide substrate for the

Scheme 15



putative enzyme. While dehydrorabelomycin is an early intermediate in the pathway, cleavage of the angular ring may occur at the quinone or the hydroquinone oxidation state, yielding **63** or **64** respectively (Scheme 15). The former should be the branch point into the nitrogen heterocycles (e.g., phenanthroviridins and jadomycins) and the oxygen heterocycles (e.g., gilvocarcins[105] and chrysomycin,[106] etc.). The latter should be the immediate precursor to the benzo[*b*]fluorene ring. These *seco* compounds are also targets in the synthesis program. The source of the second nitrogen and the mechanism of diazo formation also present important questions under study.

Probing the cosmid library from *S. murayamaensis* should provide clones that will complete the kinamycin gene cluster. In the meantime, the portion already in hand has been subcloned into four smaller fragments, and the regions hybridizing to *actI* and to *actIII* (the C-9 ketoreductase of the actinorhodin pathway[82]) have been identified. Sequencing the genes in these subclones has recently started.

VI. Acknowledgments

The author is deeply indebted to a large number of outstanding coworkers who have brought our knowledge and understanding of the fascinating kinamycin pathway to its current state. Work was initiated in 1984 by Dr. Yukiharu Sato and continued in 1986 by Dr. Pamela J. Seaton. Their efforts were followed by those of Dr. John R. Carney, Dr. Jiong Chen, Dr. Martha C. Cone, Geoffrey Fuller, Dr. Makarand P. Gore, Karen A. Halley, Awatef Hassan, Seong-Tshool Hong, Chris R. Melville, Dr. Thomas O'Hare, Dr. James Soodsma, Dr. Nuria Tamayo, and Zhiwei Tang. Professors S. Ōmura (Kitasato Institute) and U. Hornemann (University of Wisconsin) are thanked for providing strains of *S. murayamaensis*. The research has been generously supported by grant GM31715 to S. J. G. from the U. S. Public Health Service.

VII. References

(1) Ito, S.; Matsuya, T.; Ōmura, S.; Otani, M.; Nakagawa, A.; Takeshima, H.; Iwai, Y.; Ohtani, M.; Hata, T. *J. Antibiot.* **1970**, *23*, 315.
(2) Hata, T.; Ōmura, S.; Iwai, Y.; Nakagawa, A.; Otani, M. *J. Antibiot.* **1971**, *24*, 353.
(3) Ōmura, S.; Nakagawa, A.; Yamada, H.; Hata, T.; Furusaki, A.; Watanabe, T. *Chem. Pharm. Bull.* **1971**, *19*, 2428.
(4) Ōmura, S.; Nakagawa, A.; Yamada, H.; Hata, T.; Furusaki, A.; Watanabe, T. *Chem. Pharm. Bull.* **1973**, *21*, 931.
(5) Furusaki, A.; Matsui, M.; Watanabe, T.; Ōmura, S.; Nakagawa, A.; Hata, T. *Isr. J. Chem.* **1972**, *10*, 173.
(6) Sato, Y.; Geckle, M.; Gould, S. J. *Tetrahedron Lett.* **1985**, *26*, 4019.
(7) Gould, S. J.; Tamayo, N.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 2207.
(8) Mithani, S.; Weeratunga, G.; Taylor, N. J.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **1994**, *116*, 2209.
(9) Seaton, P. J.; Gould, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 5282.
(10) Rohr, J.; Thiericke, R. *Nat. Prod. Rep.* **1992**, *9*, 103.
(11) Cone, M. C.; Seaton, P. J.; Halley, K. A.; Gould, S. J. *J. Antibiot.* **1989**, *42*, 179.
(12) Isshiki, K.; Sawa, T.; Naganawa, H.; Matsuda, N.; Hattori, S.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1989**, *42*, 467.
(13) Smitka, T. A.; Bonjouklian, R.; Perun, J. T., Jr..; Hunt, A. H.; Foster, R. S.; Mynderse, J. S.; Yao, R. C. *J. Antibiot.* **1992**, *45*, 581.
(14) Lin, H.-C.; Chang, S.-C.; Wang, N.-L.; Chang, L.-R. *J. Antibiot.* **1994**, *47*, 675.
(15) Young, J.-J.; Ho, S.-N; Ju, W.-M.; Chang, L.-R. *J. Antibiot.* **1994**, *47*, 681.
(16) Ajisaka, K.; Takeshima, H.; Ōmura, S. *J. Chem. Soc. Chem. Commun.* **1976**, 571.
(17) Sato, Y.; Gould, S. J. *Tetrahedron Lett.* **1985**, *26*, 4023.
(18) Hornemann, U.; Kehrer, J. P. Commented briefly upon the kinamycin structure containing a ′*m*C_7N″ unit (ref 19).
(19) Hornemann, U.; Kehrer, J. P. *J. Am. Chem. Soc.* **1974**, *96*, 320.
(20) Birch, A. J.; Donovan, F. W. *Aust. J. Chem.* **1955**, *8*, 529.
(21) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T. *Tetrahedron Lett.* **1977**, 483.
(22) Seto, H.; Yonehara, H. *J. Antibiot.* **1977**, *30*, 1019.
(23) Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T.; Yamasaki, K. *Chem. Pharm. Bull.* **1981**, *29*, 3536.
(24) Campbell, I. M.; Robins, D. J.; Kelsey, M.; Bentley, R. *Biochemistry* **1971**, *10*, 3069.
(25) Sharf, K.-H.; Zenk, M. H.; Onderka, D. K.; Carroll, M.; Floss, H. G. *J. Chem. Soc., Chem. Commun.* **1971**, 765.
(26) Grotzinger, F.; Campbell, I. M. *Phytochemistry* **1972**, *11*, 675.
(27) White, R. J.; Martinelli, E.; Gallo, G. G.; Lancini, G. *Nature* **1973**, *243*, 273.
(28) Kibby, J. J.; McDonald, I. A.; Rickards, R. W. *J. Chem. Soc., Chem. Commun.* **1980**, 768.
(29) Seaton, P. J.; Gould, S. J. *J. Antibiot.* **1989**, *42*, 189.
(30) Liu, W.-C.; Parker, W. L.; Slusarchyk, D. S.; Greenwood, G. L.; Graham, S. F.; Meyers, E. *J. Antibiot.* **1970**, *23*, 437.
(31) Seaton, P. J.; Gould, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 5912.
(32) Fendrich, G.; Zimmermann, W.; Gruner, J.; Auden, J. A. L. Eur. Patent Appl. EP 304,400, 22 Feb 1989; *Chem. Abstr.* **1989**, *112*, 75295q.
(33) Ayer, S. W.; McInnes, A. G.; Thibault, P.; Walter, J. A.; Doull, J. L.; Parnell, T.; Vining, L. C. *Tetrahedron Lett.* **1991**, *32*, 6301.
(34) Doull, J. L.; Ayer, S. W.; Singh, A. K.; Thibault, P. *J. Antibiot.* **1993**, *46*, 869.
(35) Gore, M. P.; Gould, S. J.; Weller, D. D. *J. Org. Chem.* **1991**, *56*, 2289.
(36) Gore, M. P.; Gould, S. J.; Weller, D. D. *J. Org. Chem.* **1992**, *57*, 2774.
(37) Cone, M. C.; Hassan, A. M.; Gore, M. P.; Gould, S. J.; Borders, D. B.; Alluri, M. R. *J. Org. Chem.* **1994**, *59*, 1923.
(38) Kuntsmann, M. P.; Mitscher, L. A. *J. Org. Chem.* **1966**, *31*, 2920.
(39) Drautz, H.; Zahner, H. *J. Antibiot.* **1986**, *39*, 1657.
(40) Hayakawa, Y.; Furihata, K.; Seto, H.; Otake, N. *Tetrahedron Lett.* **1985**, *26*, 3471.
(41) Imamura, N.; Kakinuma, K.; Ikekawa, N.; Tanaka, H.; Ōmura, S. *J. Antibiot.* **1982**, *35*, 602.
(42) Rohr, J.; Beale, J. M.; Floss, H. G. *J. Antibiot.* **1989**, *42*, 1151.
(43) Dmitrienko, G. I.; Nielsen, K. E.; Steingart, C.; Ming, N. S.; Willson, J. M.; Weeratunga, G. *Tetrahedron Lett.* **1990**, *31*, 3681.
(44) Weeratunga, G.; Prasad, G. K. B.; Dilley, J.; Taylor, N. J.; Dmitrienko, G. I. *Tetrahedron Lett.* **1990**, *31*, 5713.
(45) Kobayashi, K.; Takeuchi, H.; Seko, S.; Suginome, H. *Helv. Chim. Acta* **1991**, *74*, 1091.
(46) O'Sullivan, P. J.; Moreno, R.; Murphy, W. S. *Tetrahedron Lett.* **1992**, *33*, 535.
(47) Knolker, H.-J.; O'Sullivan, N. *Tetrahedron* **1994**, *50*, 10893.
(48) Echavarren, A. M.; Tamayo, N.; Parades, M. C. *Tetrahedron Lett.* **1993**, *34*, 4713.
(49) Stoessl, A. *Can. J. Chem.* **1969**, *47*, 777.
(50) Stoessl, A. *Can. J. Chem.* **1969**, *47*, 767.
(51) Stoessl, A.; Unwin, C. H. *Can J. Chem.* **1983**, *61*, 372.
(52) Cone, M. C.; Gould, S. J. Unpublished results.
(53) Shin-ya, K.; Furihata, K.; Teshima, Y.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **1992**, *33*, 7025.
(54) Cone, M. C.; Melville, C. R.; Gore, M. P.; Gould, S. J. *J. Org. Chem.* **1993**, *58*, 1058.
(55) Aoyama, T.; Zhao, W.; Kojima, F.; Muraoka, Y.; Naganawa, H.; Takeuchi, T.; Aoyagi T. *J. Antibiot.* **1993**, *46*, 1471.
(56) Gould, S. J.; Gore, M. P. Unpublished results.
(57) Gould, S. J.; Melville, C. R.; Cone, M. C.; Chen, J.; Carney, J. R. *J. Org. Chem.* **1997**, in press.
(58) Gould, S. J.; Melville, C. R. Unpublished results.
(59) Gould, S. J.; Melville, C. R. *Bioorg. Med. Chem. Lett.* **1995**, *6*, 51.
(60) Hauser, F. M.; Zhou, M. *J. Org. Chem.* **1996**, *61*, 5722.
(61) Gould, S. J.; Chen, J.; Cone, M. C.; Gore, M. P.; Melville, C. R.; Tamayo, N. *J. Org. Chem.* **1996**, *61*, 5720.
(62) Gould, S. J.; Chen, J. Unpublished results.
(63) Wang, W.; Snieckus, V. *J. Org. Chem.* **1992**, *57*, 424.
(64) Mal, D.; Hazra, N. K. *Tetrahedron Lett.* **1996**, *37*, 2641.
(65) Suttie, J. W.; McTigue, J.; Larson, A. E.; Wallin, R. *Ann. N. Y. Acad. Sci.* **1981**, 271.
(66) Kuliopulos, A.; Hubbard, B. R.; Lam, Z.; Koski, I. J.; Furie, B.; Furie, B. C.; Walsh, C. T. *Biochemistry* **1992**, *31*, 7722.
(67) Dowd, P.; Hershline, R.; Ham, S. W.; Naganathan, S. *Science* **1995**, *269*, 1684.
(68) Gould, S. J.; Shen, B.; Whittle, Y. G. *J. Am. Chem. Soc.* **1989**, *111*, 7932.
(69) Shen, B.; Gould, S. J. *Biochemistry* **1991**, *30*, 8936.

(70) Gould, S. J.; Kirchmeier, M. J.; LaFever, R. E. *J. Am. Chem. Soc.* **1996**, *118*, 7663.
(71) Gould, S. J.; O'Hare, T.; Seaton, P.; Soodsma, J.; Tang, Z. *Bioorg. Med. Chem.* **1996**, *4*, 987.
(72) Nakano, H.; Matsuhashi, Y.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1977**, *30*, 76.
(73) Perez-Gonzalez, J. A.; Vara, J.; Jimenez, A. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 772.
(74) Shaw, V. W. *CRC Crit. Rev. Biochem.* **1983**, *14*, 1.
(75) Paik, S.-Y.; Sugiyama, M.; Nomi, R. *J. Antibiot.* **1985**, *38*, 1761.
(76) Sugiyama, M.; Takeda, A.; Paik, S.-Y.; Mini, O.; Nomi, R. *J. Antibiot.* **1986**, *39*, 827.
(77) Thompson, C. J.; Movva, N. R.; Tizard, R.; Crameri, R.; Davies, J. E.; Lauwereys, M.; Botterman, J. *EMBO J.* **1987**, *6*, 2519.
(78) Kobayashi, T.; Horinuchi, S.; Uozumi, T.; Beppu, T. *J. Antibiot.* **1987**, *40*, 1016.
(79) Botterman, J.; Gossele, V.; Thoen, C.; Lauwereys, M. *Gene* **1991**, *102*, 33.
(80) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37.
(81) Malpartida, F.; Hallam, S. E.; Kieser, H. M.; Motamedi, H.; Hutchinson, C. R.; Butler, M. J.; Sugden, D. A.; Warren, M.; McKillop, C.; Bailey, C. R.; Humphreys, G. O.; Hopwood, D. A. *Nature* **1987**, *325*, 818.
(82) Fernandez-Moreno, M. A.; Martinez, E.; Boto, L.; Hopwood, D. A.; Malpartida, F. *J. Biol. Chem.* **1992**, *267*, 19278.
(83) Han, L.; Yang, K.; Ramalingam, E.; Mosher, R. H.; Vining, L. C. *Microbiology* **1994**, *140*, 3379.
(84) Yang, K.; Han, L.; Ayer, S. W.; Vining, L. C. *Microbiology* **1996**, *142*, 123.
(85) Decker, H.; Haag, S. *J. Bacteriol.* **1995**, *177*, 6126.
(86) Hong, S.-T.; Carney, J. R.; Gould, S. J. *J. Bacteriol.* **1997**, *179*, 470.
(87) Wilton, J. H.; Cheney, D. C.; Hokanson, G. C.; French, J. C. *J. Org. Chem.* **1985**, *50*, 3936.
(88) Gould, S. J.; Cheng, X.-C.; Halley, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 10066.
(89) Gould, S. J.; Cheng, X.-C.; Melville, C. R. *J. Am. Chem. Soc.* **1994**, *116*, 1800.
(90) Tsai, J. F.-Y.; Chen, C. W. *Mol. Gen. Genet.* **1987**, *208*, 211.
(91) Zhou, X.; Deng, Z.; Hopwood, D. A.; Kieser, T. *Mol. Microbiol.* **1994**, *12*, 789.
(92) Gould, S. J.; Hong, S.-T.; Carney, J. R. Submitted for publication.
(93) Carney, J. R.; Hong, S.-T.; Gould, S. J. *Tetrahedron Lett.* **1997**, *38*, 3139.
(94) Sato, Y.; Kohnert, R.; Gould, S. J. *Tetrahedron Lett.* **1986**, *27*, 143.
(95) Gould, S. J.; Melville, C. R.; Chen, J. *Tetrahedron* **1997**, *13*, 4561.
(96) Carney, J. R.; Hassan, A. M.; Cone, M. C.; Gould, S. J. Unpublished results.
(97) Hassan, A. M.; Carney, J. R.; Gould, S. J. Unpublished results.
(98) Cone, M. C.; Carney, J. R.; Gould, S. J. Unpublished results.
(99) Hassan, A. M.; Cone, M. C.; Melville, C. R.; Gould, S. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 191.
(100) French, J. C. Personal communication.
(101) Melville, C. R.; Gould, S. J. Unpublished results.
(102) Cone, M. C.; Melville, C. R.; Carney, J. R.; Gore, M. P.; Gould, S. J. *Tetrahedron* **1995**, *51*, 3095.
(103) Gould, S. J.; Melville, C. R.; Cone, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 9228.
(104) Hu, Y.; Melville, C. R.; Gould, S. J.; Floss, H. G. *J. Am. Chem. Soc.* **1997**, *119*, 4301.
(105) Takahashi, K.; Yoshida, M.; Tomita, F.; Shirahata, K. *J. Antibiot.* **1981**, *34*, 271.
(106) Carter, G. T.; Fantini, A. A.; James, J. C.; Borders, D. B.; White, R. J. *J. Antibiot.* **1985**, *38*, 242.

CR9600215