Elucidation and Formulation of Novel Biosynthetic Pathways Leading to the Pyrrolo[1,4]benzodiazepine Antibiotics Anthramycin, Tomaymycin, and Sibiromycin

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"When you have eliminated the impossible, whatever remains, however improbable, must be the truth."

Sir Arthur Conan Doyle (1859–1930)

Antibiotics often occur in groups of closely related compounds, presumably because they are derived via similar biosynthetic pathways, but late biosynthetic events give rise to a multiplicity of products. The later biosynthetic events may be termed "cosmetic after events" and could well result from the presence of enzymes which have low specificities and perhaps other primary functions in the antibiotic producing cells, although definitive information on this point is lacking. In some cases the multiplicity of products may be found within the same organism, for example, the tetracyclines,[1] neomycins,[2] and bleomycins.[3] In other cases, while only single antibiotics within one group appear to be produced, biosynthetic analogues are produced by other organisms. This is the case with the "C-7 unit" found in mitomycin C,[4] validamycin A,[5] kinamycin C,[6] rifamycin S,[7] and geldanamycin.[8]

We have determined the biosynthetic precursors and their labeling patterns in the pyrrolo[1,4]benzodiazepine antibiotics anthramycin (I), tomaymycin (II), and sibiromycin (III). The results show that they have biosynthetic analogies in the antibiotics actinomycin D and lincomycins A (Va) and B (Vb). Tryptophan is the common biosynthetic precursor of the anthranilate moieties of actinomycin D and the pyrrolo[1,4]benzodiazepine antibiotics, while tyrosine is the common precursor of the C_2 and C_3 proline units of the lincomycins and the pyrrolo[1,4]benzodiazepine antibiotics. Intriguingly, whereas in the case of the pyrrolo[1,4]-benzodiazepine group only a C_2 *or* C_3 proline unit is produced, in the case of the lincomycins both C_2 *and* C_3 proline units are produced side by side in this fermentation.

It is the main purpose of this Account to illustrate how we have elucidated and then consolidated the biosynthetic information from our studies on the members of the pyrrolo[1,4]benzodiazepine with that previously determined on actinomycin D and the lincomycins.

The biosynthetic labeling patterns of precursors in the pyrrolo[1,4]benzodiazepine antibiotics were determined using state-of-the-art techniques such as ^1H and ^{13}C NMR in combination with ^2H, ^{13}C, and ^{15}N specifically labeled compounds. This biosynthetic information, together with the relative retentions of various tritium atoms from precursors of the various antibiotics, has allowed us to formulate logical biosynthetic grids leading from tryptophan and tyrosine to the anthranilate and C_2 or C_3 proline units of the various antibiotics.

The pyrrolo[1,4]benzodiazepine antibiotics are of considerable interest to us not only because of their unique biosynthetic origin but also because they are very potent antitumor agents with a unique mechanism of action.[9] Anthramycin and related drugs form a labile covalent adduct with DNA[10–13] which results in inhibition of nucleic acid synthesis[14,15] and, at least in the case of anthramycin, excision repair of DNA,[16] recombinogenic effects in yeast,[17] and sister chromatid exchange in skin fibroblasts.[18] We have recently proposed structures for the anthramycin,[19] tomaymycin, sibiromycin, and neothramycin A (IVa) and B (IVb)[20] ad-

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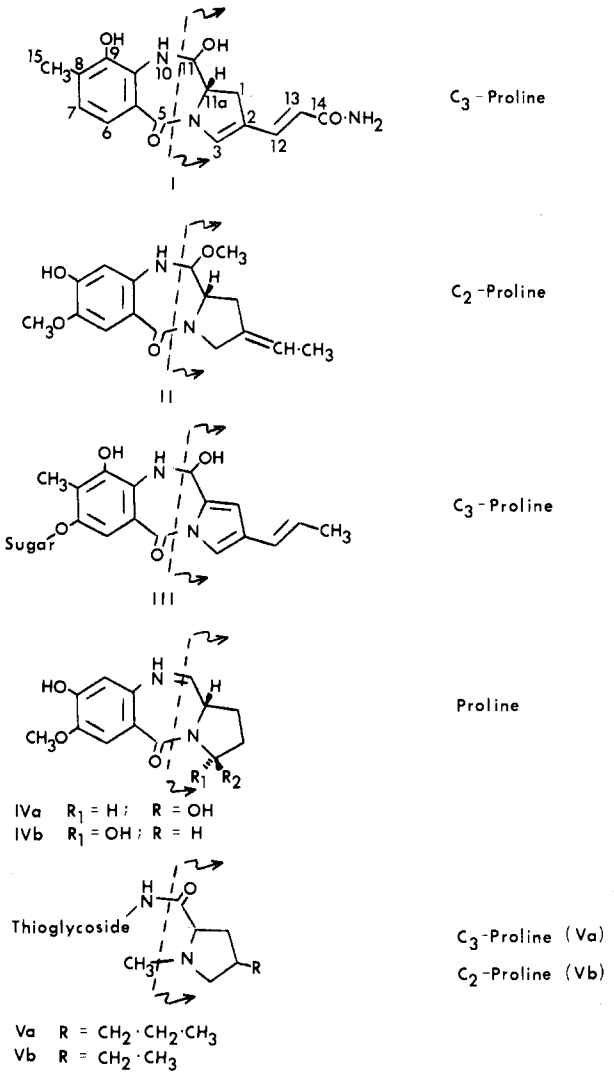

Figure 1. Structure of anthramycin (I), tomaymycin (II), sibiromycin (III), neothramycins A (IVa) and B (IVb), and lincomycin A (Va) and B (Vb).

ducts with DNA in which these drugs bind covalently through N-2 of guanine and lie snugly hidden in the narrow groove of DNA.

Speculation and Confirmation of the Biosynthetic Precursors

The structures of the pyrrolo[1,4]benzodiazepine antibiotics are shown in Figure 1. My interest in the biosynthesis of these structurally unique groups of compounds arose primarily from the unusual C_2 and C_3 proline units of anthramycin, tomaymycin, and sibiromycin (see Figure 1), since it seemed highly likely that the anthranilate moieties were derived from tryptophan via the kynurenine pathway.[21] Pen and paper chemistry led to a number of attractive but later proven erroneous ideas. For example, proline plus a C_3 unit, or two condensed molecules of δ-aminolevulinic acid, seemed reasonable for the acrylamide proline unit of anthramycin, or, if all else failed, an acetate/propionate combination seemed a plausible option. Upon the gentle suggestion of a colleague at Purdue we checked a paper published by Witz and co-workers on the biosynthesis of the lincomycins[22] which provided evidence that tyrosine, of all things, was a likely precursor of the "C_2 and C_3 proline" units of these antibiotics. I was both relieved and also quite elated when we demonstrated very shortly afterwards that tyrosine was indeed a very efficient precursor.[23] This finding was exciting to me because now I had the intriguing problem of elucidating how tyrosine was converted to the quite structurally varied C_2 and C_3 proline units of anthramycin, tomaymycin, and the recently reported sibiromycin. The neothramycins (IVa and IVb) were not reported until 1976, which was perhaps fortunate for us, since recently Miyamoto[24] has shown the "proline" unit of these antibiotics is derived in an unexceptional way from L-proline and this might well have discouraged us from initiating this work.

Our demonstration that tyrosine and Dopa were biosynthetic precursors of anthramycin was followed by experiments in which we are able to show that tryptophan radiolabeled in the aromatic ring and methionine radiolabeled in the *S*-methyl group were also very efficiently incorporated. Our experiments with the anthramycin-producing strain were greatly facilitated because the producing organism grows at 47 °C and the complete fermentation takes only about 12 h. A biosynthetic scheme for the conversion of tyrosine, tryptophan, and methionine into the pyrrolo[1,4]benzodiazepine antibiotics is shown in Figure 2.

The strategy which follows in this Account provided us with answers on the following: (1) how tryptophan is biosynthetically converted to the anthranilic acid moieties of the pyrrolo[1,4]benzodiazepines and the probable order of insertion of substituents into the aromatic ring; (2) how the carbon skeleton of tyrosine becomes a C_2 or C_3 proline unit; and (3) how the diversity in C_2 and C_3 proline moieties might be explained based upon a rational "biosynthetic grid".[25] I use the term "biosynthetic grid" to describe a main arterial pathway leading from tyrosine to a key branch point compound, from which then follow, in some cases, parallel but divergent pathways to the C_2 proline unit of lincomycin B and tomaymycin and to the C_3 proline units of lincomycin A, anthramycin, and sibiromycin.

Tryptophan as the Precursor of the Anthranilate Units of Anthramycin, Tomaymycin, and Sibiromycin

The occurrence of 4-methyl-3-hydroxyanthranilic acid units in actinomycin D and anthramycin led us to suspect that they were derived via similar pathways from tryptophan, the established precursor of this unit in actinomycin D.[21] Feeding experiments with DL-[7a-^{14}C]tryptophan and [*methyl*-^{14}C]methionine followed by chemical degradation of the biosynthetically labeled anthramycin provided proof that the radioactivity from the tryptophan was found exclusively in the anthra-

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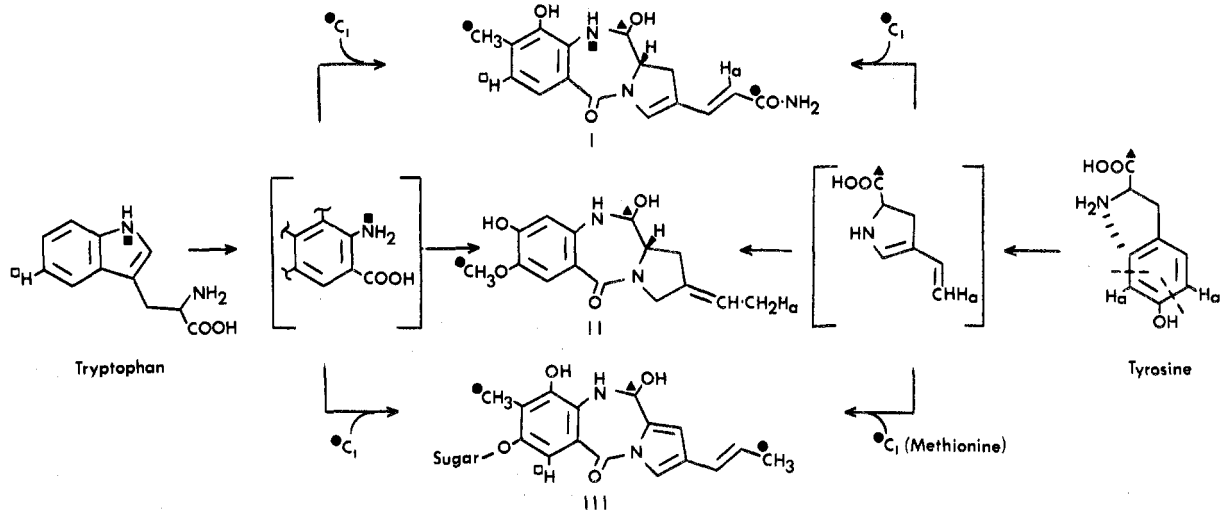

Figure 2. Biosynthetic conversion of tryptophan, tyrosine, and methionine into the pyrrolo[1,4]benzodiazepine antibiotics.

nilate unit while the label from methionine was found in the aromatic methyl group and also in the acrylamide proline moiety.[23] Similar experiments using DL-[7a-^{14}C]tryptophan with the tomaymycin,[27a] sibiromycin,[27b] and neothramycins A and B[24] producing organisms showed analogous results.

The manner in which tryptophan is incorporated into the anthranilate ring of anthramycin was determined by location of the ^{15}N atom from L-(*indole*-^{15}N)tryptophan in the anthramycin molecule. The indole nitrogen of tryptophan was found to reside exclusively at N-10 of anthramycin by observation of the clear coupling of a ^{13}C-enriched carbon at C-11 of anthramycin with the adjacent ^{15}N atom in the ^{13}C NMR spectra of the dual label (^{15}N and ^{13}C) enriched antibiotic.[28] Since tritium from DL-[5-^{3}H]tryptophan is completely retained in anthramycin, this leads to the unambiguous labeling pattern of tryptophan shown in Figure 2.[29]

The availability of DL-[5-^{3}H]tryptophan and a knowledge of NIH shift rules[30] have allowed us to predict the order in which substituents are introduced into the aromatic ring of tomaymycin and sibiromycin by determination of tritium retentions in each of these antibiotics.[29] In the case of tomaymycin a very low retention of tritium suggests that the biosynthetic pathway involves hydroxylation at C-8 prior to hydroxylation at C-7 since, according to NIH shift rules,[30] hydroxylation ortho to an existing hydroxyl group leads to loss of tritium. However, in the case of sibiromycin the almost complete retention of tritium (93%) from DL-[7a-^{14}C;5-^{3}H]tryptophan demonstrates that an NIH shift occurs and that this hydroxylation takes place on a compound possessing a less activated para substituent such as an amide. Other results from competition experiments between unlabeled kynurenine derivatives and carbon-14 labeled tryptophan indicate that 9-hydroxylation and 8-methylation precede this NIH shift.[31]

Establishment of the Precursors and Their Biosynthetic Labeling Pattern in the C_2 and C_3 Proline Units of Anthramycin, Tomaymycin, and Sibiromycin

By far the most intriguing aspect of our biosynthetic work was elucidation of the manner in which tyrosine is converted to the C_2 and C_3 proline units of anthramycin, tomaymycin, and sibiromycin. Conceivably, parallel pathways leading to the C_2 proline (C_7) or C_3 proline (C_8) units could arise either from a common ring-cleavage product, with (C_8) or without (C_7) a C_1 unit, or ring cleavage products differing by one carbon but each receiving a similar C_1 unit from methionine. The earlier work by the Upjohn group on the lincomycins suggested the latter sequence;[22] however, our subsequent results on anthramycin,[23] tomaymycin,[27a] and sibiromycin[27b] and then later experiments by the Upjohn group[32] refuted this in favor of the former hypothesis involving only a common ring-cleavage reaction, with or without an additional C_1 unit.

Critical and definitive experiments utilizing variously labeled tyrosine and methionine molecules were carried out to determine (1) how many carbon atoms were derived from tyrosine, (2) whether methionine labeled the C_2 or C_3 proline units, and (3) the precise labeling patterns of tyrosine and methionine in these units. Tyrosine contributed exactly seven carbon atoms to each antibiotic molecule, since L-[U-^{14}C]tyrosine was incorporated only $^{7}/_{9}$ as efficiently as L-[1-^{14}C]tyrosine.[23,27] The terminal carbon atoms (C-14) of anthramycin and sibiromycin are derived from the C_1 pool via methionine, whereas the ethylidene methyl group of tomaymycin is derived from carbon atom 3 or 5 of the aromatic ring of tyrosine. These findings were based upon a ^{13}C NMR examination of L-[*methyl*-^{13}C]-methionine-enriched anthramycin[23] and sibiromycin[27b] and chemical degradation (Kuhn–Roth oxidation)[33] of L-[3- or 5-^{3}H]tyrosine-labeled tomaymycin.[27a] It remained to pinpoint the exact location of the side-chain carbons and hydrogens of tyrosine and the other aromatic hydrogens in each of the antibiotics. This we did

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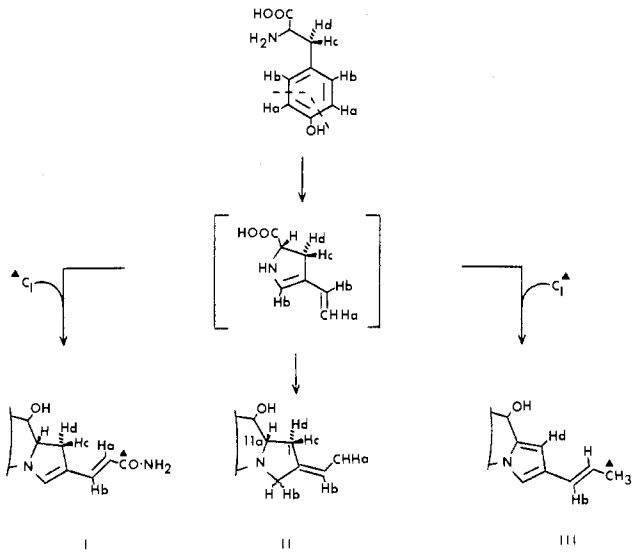

Figure 3. Biosynthetic labeling pattern of tyrosine in the C_2 and C_3 proline units of anthramycin (I), tomaymycin (II), and sibiromycin (III).

by chemically degrading samples of anthramycin labeled from L-[(3′-*RS*)-3′-^{3}H]tyrosine[34] or by ^{13}C NMR or ^{1}H NMR examination of anthramycin biosynthetically labeled from L-[1′-^{13}C]tyrosine[23] (^{13}C NMR), L-[3- and 5-$^2H_2$]tyrosine[23] (^{1}H NMR), and L-[2- and 6-$^2H_2$]-tyrosine[35] (^{1}H NMR). In many cases this was repeated for samples of sibiromycin[27b] and tomaymycin.[27a] A summary of the proven biosynthetic labeling patterns is shown in Figure 3.

At this point in our biosynthetic work we were cognizant of how the carbon atoms of tyrosine provided the C_2 and C_3 proline skeletons of the pyrrolo[1,4]benzodiazepine antibiotics, but we had little understanding of the sequence of biosynthetic reactions and therefore the identity of the intermediates. This objective was therefore our next step.

Relative Retentions of Aromatic and Side-Chain Hydrogens from Tyrosine in the C_2 and C_3 Proline Units of Anthramycin, Tomaymycin, and Sibiromycin

During conversion of tyrosine to the C_2 and C_3 proline units of the pyrrolo[1,4]benzodiazepine antibiotics, a number of biosynthetic events must take place, including aromatic ring cleavage, cyclization to form the pyrrolo ring, loss of two aromatic carbons, and, in the case of sibiromycin, loss of one of the two diastereotopic 3′-hydrogens and the 2′-hydrogen of tyrosine. The availability of specifically tritiated tyrosine molecules, together with their vigorously established biosynthetic labeling patterns in each of the antibiotics, has allowed us to interpret the relative tritium retentions in terms of possible biosynthetic intermediates between tyrosine and the final biosynthetic products. In general, this strategy has allowed us to eliminate many alternatives which are incompatible with the data while leaving open a few options which must then be chosen between. Where choice between various options still exists, we have chosen the option that is most mechanistically reasonable while bearing in mind that there is probably

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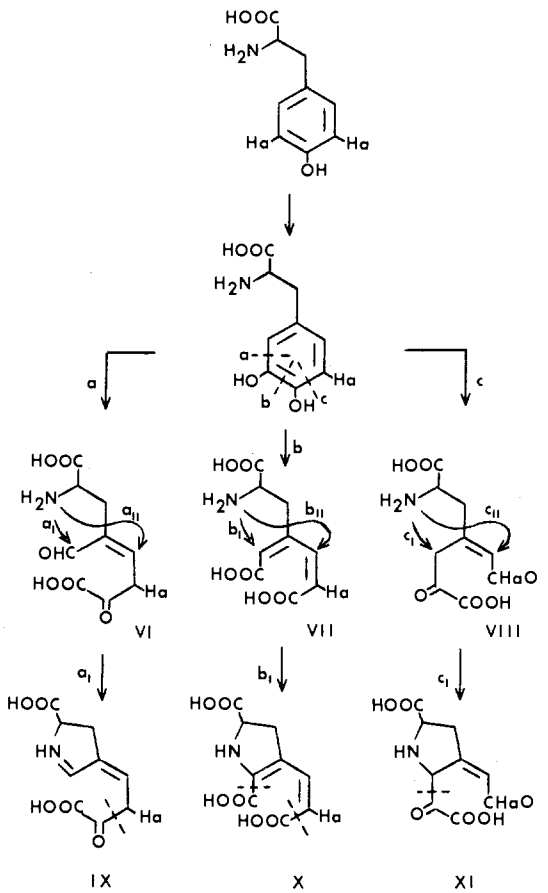


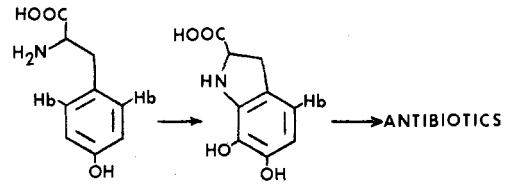
Figure 4. Alternative pathways for ring cleavage and cyclization of Dopa.

a main arterial pathway leading through a key branch-point intermediate to parallel biosynthetic pathways to the C_2 and C_3 proline units of pyrrolo-[1,4]benzodiazepine and the lincomycin antibiotics.

Implications of the Relative Retention of Tritium, from L-[3- or 5-^{3}H]Tyrosine in the Pyrrolo[1,4]benzodiazepine Antibiotics, for the Type of Aromatic Ring Cleavage Reaction

During conversion of L-[3- or 5-^{3}H]tyrosine to the C_2 and C_3 proline units, *at least* 50% of the tritium must be lost during the tyrosine hydroxylase reaction (see Figure 4). However, the subsequent loss or retention of the remaining 50% of the tritium will depend upon both the type of aromatic ring cleavage and cyclization reactions and the subsequent "cosmetic after events" occurring at the carbon atom carrying the tritium atom.

Dopa can undergo three types of ring cleavage (a, b, and c in Figure 4), and each of these products can then cyclize in two possible ways (a_1, a_2; b_1, b_2; c_1, c_2; Figure 4), providing a total of six alternative pathways. Three of these six pathways (a_2, b_2, and c_2) would necessitate *complete loss* of tritium from L-[3- or 5-^{3}H]tyrosine in the final antibiotics, and since in the cases of anthramycin[23] and tomaymycin[27a] a 50% retention of tritium is found, these alternatives can be eliminated, at least for these two antibiotics. Figure 4 illustrates *only* the viable alternatives (a_1, b_1, and c_1) which follow proximal extradiol cleavage (a), intradiol cleavage (b), and distal extradiol cleavage (c), respectively. The choice of the most likely of these three remaining viable pathways shown in Figure 4 is based upon the occurrence in the fermentation broths of all three antibiotic producing



XII

Figure 5. Cyclodopa (XII) as an intermediate in the biosynthesis of the pyrrolo[1,4]benzodiazepine antibiotics.

strains of a bright yellow acidic compound which has spectral characteristics of the aldehydo oxo acid VI. This aldehydo oxo acid has been synthesized recently[36] and has been found to cyclize to a dihydropyrrole derivative[36] (IX in Figure 4) and muscaflavin, a fungal pigment.[37]

We therefore strongly suspect that a proximal extradiol cleavage of Dopa followed by spontaneous ring closure to form the Schiff base (IX in Figure 4) is the common pathway to these antibiotics. Quite unexpectedly we found that sibiromycin loses virtually all of the tritium from L-[3- or 5-^{3}H]tyrosine;[27b] however, we believe this is due to a later "cosmetic after event" which is linked indirectly to the extra degree of unsaturation found only in the pyrrolo ring sibiromycin.

Retention of L-[2- or 6-^{3}H]Tyrosine in Anthramycin, Tomaymycin, and Sibiromycin and the Timing of the Cyclization Reaction

The intermediacy of cyclodopa (XII) requires that at least 50% of tritium should be lost from L-[2- or 6-^{3}H]tyrosine during its conversion to the pyrrolo[1,4]benzodiazepine antibiotics (see Figure 5). Our results show that in the case of tomaymycin (78% retention tritium)[27a] cyclodopa cannot be a viable intermediate, and therefore ring cleavage takes place *prior* to formation of the five-membered ring. The 50% and 32.7% retentions of tritium in anthramycin[23] and sibiromycin,[27b] respectively, would appear to leave open this possibility. However, in accord with the concept that there is a common main pathway ending in a branch-point compound which itself leads to either the C_2 or C_3 proline moieties of these antibiotics by "cosmetic after events", we feel that the loss of greater than 50% of the tritium in these experiments, using L-[2- or 6-^{3}H]tyrosine, is most likely due to a later biosynthetic event rather than the intermediacy of cyclodopa.

Retentions of the Side-Chain Hydrogens of Tyrosine in Anthramycin, Tomaymycin, and Sibiromycin

Examination of the side-chain carbons of tyrosine and their biosynthetic fate in the pyrrolo[1,4]benzodiazepine antibiotics reveals that, whereas in anthramycin and tomaymycin retention at carbon atoms 11a and 1 of both C-2′ and C-3′ hydrogens of tyrosine is possible, in the case of sibiromycin the 2′-hydrogen and one of the two diastereotopic 3′-hydrogens must be lost (see Figure 3). Using a combination of double-labeled tyrosine molecules, including the two stereospecifically C-3′ tritiated species, we have shown that, while in anthramycin and tomaymycin retention of both C-3′ hydrogens occurs, in sibiromycin there is a predominant stereospecific loss of the 3′(*S*)-hydrogen of tyrosine.[35]

The fate of the hydrogen at C-2′ of tyrosine in the three antibiotics was determined by using L-[1′-^{14}C;-Ala-2′- or 3′-^{3}H]tyrosine, since the tritium labeling pattern in the amino acid is known,[38] and we had already established the fate of the C-3′ hydrogens of tyrosine. Since the stereochemistry at C-2′ of tyrosine is the same as that at C-11a of anthramycin and tomaymycin, we were somewhat surprised to find complete loss of the C-2′ hydrogen in both of these antibiotics as well as in sibiromycin.[35] The possibility that either racemization or a transaminase was responsible for this complete loss of tritium at C-2′ of tyrosine was explored in competition experiments between L- and D-tyrosine and experiments with DL-[1′-^{14}C;3- and 5-$^2H_2$;^{15}N]tyrosine, respectively. L-Tyrosine was found to be the specific precursor of all the pyrrolo[1,4]-benzodiazepine antibiotics using L-[2- or 6-^{3}H]tyrosine and D-[2- or 6-^{3}H]tyrosine, with DL-[1′-^{14}C]tyrosine as a reference label in experiments similar to that originally proposed by Leistner et al.[39] to distinguish between different isomer specific utilization. Although only a partial retention of ^{15}N from DL-[^{15}N]tyrosine was found in anthramycin (22%) and sibiromycin (29%) compared to the deuterium or ^{14}C retention, this is unlikely to be entirely responsible for the complete loss of the 2′-hydrogen of tyrosine in anthramycin.[35] The reason for the complete loss of the C-2′ hydrogen of tyrosine in anthramycin and sibiromycin is therefore at this time unknown.

Formulation of the Biosynthetic Grid from Tyrosine to the C_2 and C_3 Proline Units of the Pyrrolo[1,4]benzodiazepine and Lincomycin Antibiotics

On the basis of a detailed examination of the conversion of tyrosine to the C_2 and C_3 proline units of the pyrrolo[1,4]benzodiazepine antibiotic, a biosynthetic grid has been proposed.[35] This grid, consisting of a common main pathway leading to a branch-point compound, then diverges into five branches, each leading ultimately to one of the C_2 and C_3 proline units of the pyrrolo[1,4]benzodiazepine or lincomycin antibiotics. Not only does this proposed grid accommodate all our data, but it also explains some of the apparent inconsistencies which appear between experiments using the same isotopically labeled precursor and the different antibiotics.

This pathway is shown in Figure 6 and is based upon the following assumptions: (1) proximal extradiol cleavage of Dopa is involved as the common ring-cleavage reaction; (2) cyclodopa is unlikely to be a common intermediate in the pathway; (3) divergence of the pathways occurs at the step at which addition of either a CH_3^+ group or a H^+ to a common C_2 proline unit takes place; and (4) formation of the ethylidene methyl group of tomaymycin and the conjugated acrylamide side chain of anthramycin and the unsaturation in the side chain and pyrrolo ring of sibiromycin are cosmetic after events which occur subsequent to the main pathway.

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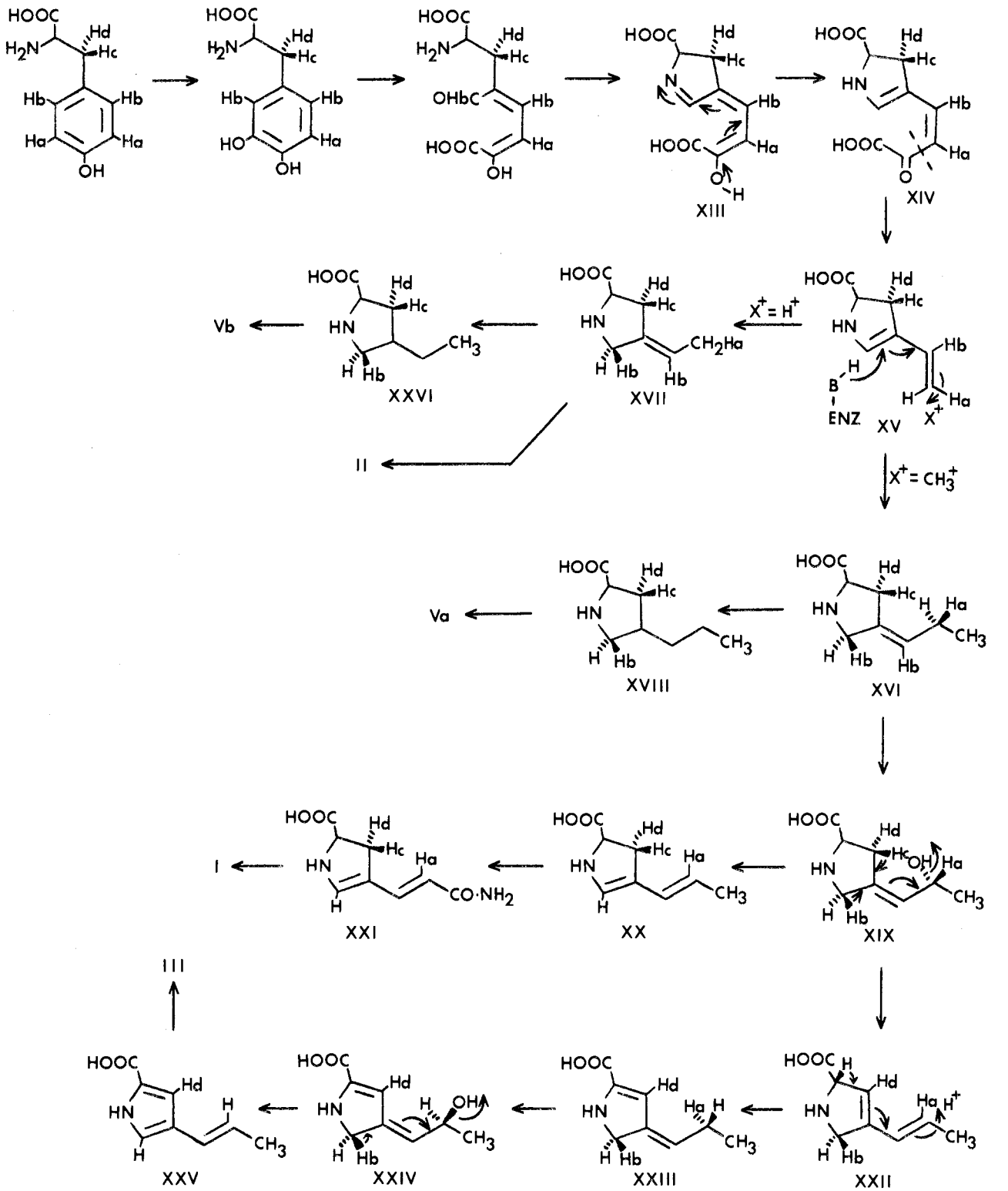

Figure 6. Proposed biosynthetic grid for the conversion of tyrosine into the C_2 and C_3 proline units of anthramycin (I), tomaymycin (II), sibiromycin (III), and lincomycins A (Va) and B (Vb).

The important features of the main and branch pathways leading to the C_2 and C_3 proline units of the pyrrolo[1,4]benzodiazepine antibiotics and the lincomycins shown in Figure 6 are as follows: (1) Following 2,3-extradiol cleavage of the aromatic ring of Dopa, a condensation reaction to form a Schiff base between the α-amino group and the aldehydic group takes place. (2) The conjugated enol XIII then undergoes tautomerization to yield the α-keto acid XIV which itself then loses two carbon atoms (carbon atoms 4 and 5 of Dopa) in a stepwise manner to form the diene XV. (3) Diene XV is considered to be the branch-point compound for which a 1,4 addition of H–X results in divergent pathways dependent upon the nature of X^+. If X^+ is H^+, then the pathway leads to the C_2 proline moieties of tomaymycin and lincomycin B, whereas if X^+ is S^+CH_3, then the pathway leads to the C_3 proline moieties of anthramycin, sibiromycin, and lincomycin A. (4) Subsequent modification of the first intermediates past the branch point leads to the propylproline unit of linco-

mycin A (XVI → Va), the acrylamidoproline unit of anthramycin (XVI → I), the propylideneproline unit of sibiromycin (XVI → III), and the ethylproline unit of lincomycin B (XVII → Vb). The ethylideneproline unit of tomaymycin is formed directly from the branch-point compound XV. Where modifications are required, these would be considered as "cosmetic after events" which occur subsequent to the main pathway. The cosmetic modifications leading to the lincomycins are straightforward and do not require further comment. For the branch pathways leading to the C_3 proline units of anthramycin and sibiromycin, hydroxylation at the allylic carbon in a stereospecific manner leads to XIX. This compound can then undergo a 1,4-conjugate elimination of phosphoric acid in two analogous but different ways (XIX → XX and XIX → XXII). These reactions would result in the stereospecific loss of hydrogen that was originally at C-2 or -6 of tyrosine (anthramycin pathway) or the 3′-*S* position of tyrosine (sibiromycin pathway). The conversion of XX to XXI requires oxidation and amination to produce the acrylamidoproline moiety of anthramycin. In the case of the sibiromycin branch, XXII undergoes an allylic rearrangement to produce XXIII which is then hydroxylated at the allylic carbon, thereby eliminating in a stereospecific manner the hydrogen that was originally located at C-3 or -5 of tyrosine. The product of this reaction, XXIV, is then able to undergo a second stereospecific 1,4-conjugate elimination of phosphoric acid which leads to loss of the hydrogen originally located at C-2 or C-6 of tyrosine and concomitantly the formation of the desired propylidene side chain of sibiromycin.

A consideration of the details in Figure 6 allows us to explain the apparent inconsistencies between results from different antibiotic fermentations.

For example, the 1,4-conjugate elimination reactions (XIX → XX and XXIV → XXV) peculiar to the anthramycin and sibiromycin pathways explain the loss of the tritium originally located at C-6 of Dopa, which is at least partially retained in tomaymycin.

Furthermore, the complete loss of tritium from L-[3- or 5-^{3}H]tyrosine peculiar to the sibiromycin biosynthesis is linked indirectly to the introduction of the extra degree of unsaturation found solely in sibiromycin (11a–1 bond), which requires an additional postulated allylic hydroxylation at C-13 (XXIII → XXIV).

Although the proposed biosynthetic pathway shown in Figure 6 is probably not the only one capable of explaining our results, we feel its development and existence is an important step in our program to establish a general biosynthetic pathway to these interesting antibiotics.

Summary and Future Directions for Research

The biosynthetic work so far completed has allowed us to elucidate the building precursors and their labeled patterns in anthramycin, tomaymycin, and sibiromycin. A rational biosynthetic grid leading from tyrosine to the C_2 and C_3 proline units of the pyrrolo[1,4]benzodiazepine and lincomycin antibiotics that accounts for some unexpected results has been proposed. The next logical step in our biosynthetic project is to test the accuracy of this grid by synthesis of the appropriate postulated intermediates in labeled form. Such studies are in progress. Projects are also under way to examine how the producing organisms avoid the toxicity of these potent cytotoxic agents and also determine the biological function of any of these antibiotics in the producing cells. Intriguingly the mechanism of action of anthramycin and related drugs appears to be at least as unique as their biosynthetic origin. The characterization of the precise manner in which these drugs react with DNA could lead to significant developments in the design of new antitumor agents belonging to this class of agents.

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