of inhibition (mm) vs $\log_2$ concn (μg/mL).

Purification of Capreomycins IA and IB. The fermentation (200 mL) was incubated at 30 °C, 250 rpm for 6 days, the culture then filtered through Celite, and the filter cake washed with water. Activated charcoal (9.0 g) was added to the combined filtrate and washings and the mixture allowed. Activated charcoal (9.0 g) was added to the combined filtrate and washings and the mixture allowed to stand for ca. 30 min. The mixture was filtered through Celite and the filtrate discarded. Successive washings of the filter cake with water (60 mL) and 0.05 M HCl (60 mL) were also discarded. The filter cake was then eluted with acidic aqueous acetone (200 mL of acetone and 4.25 mL of concd HCl per L of deionized water) in a period of 15 min. The acetone eluate was concentrated in vacuo to a small volume (ca. 8 mL) and then transferred, with stirring, to acetone (10 times the volume of the concentrate) and the mixture allowed to stand in a cold room (4 °C) overnight. The acetone was decanted, and the resulting reddish-brown oily precipitate was dissolved in 6 M HCl (3 mL). This was filtered into stirred MeOH (36 mL), and the precipitate was then collected.

A column of Amberlite CG-50 (100–200 mesh, NH_4^+) (1.0 × 42.5 cm) was allowed to equilibrate in 0.4 M NH_4OAc, buffered to pH 9.0, at 4 °C. The sample (typically 450 mg) was applied to the column in a small volume (ca. 3 mL) of the buffer. The resin was eluted first with a gradient from 0.4 to 0.8 M NH_4OAc (225 mL each) and then isocratically with 0.8 M NH_4OAc (300 mL). Fractions (ca. 5 mL) were collected at 25-min intervals and were monitored by UV (280 and 254 nm). On the basis of the UV profile, fractions containing either IA or IB were combined and concentrated on a rotary evaporator to a small volume. Capreomycin IB typically began to appear in fraction 20 and IA typically began to appear in fraction 34. The concentrates were desalted separately with a column (3.0 × 10.5 cm) of Sephadex G-10 eluted with water. Capreomycin IA: mp 244–248 °C dec (lit.[9] mp 246–248 °C dec), $[\alpha]_D$ -19.6° (lit.[9] $[\alpha]_D$ -21.9°). Capreomycin IB: mp 256–259 °C dec (lit.[9] mp 253–255 °C dec), $[\alpha]_D$ -43.6° (lit.[9] $[\alpha]_D$ -44.6°).

Incorporation of [2,3,3,5,5-$^2H_5$]Arginine, 8a. A mixture of **8a**·HCl[32] (60.1 mg, 279 μmol, ≥95% ^2H at each position) and DL-[1-^{14}C]arginine (11.42 μCi) in water (10 mL) was fed in three equal portions to a 200-mL production culture 12, 32, and 56 h after inoculation with a seed culture. After 6 days the fermentation was worked up, and bioassay indicated 972 mg of total capreomycins. The MeOH precipitate (394 mg) was chromatographed on a CG-50 column (1.5 × 46 cm), eluting as described above; 8.5 mL fractions were taken.

The combined IB (**2a**) fractions were desalted on a Sephadex G-10 column (4.6 × 10.5 cm), and center fractions containing pure **2a** were lyophilized and combined to give 56 mg. A portion of this (31.1 mg) was dissolved in deuterium-depleted water, lyophilized, and dissolved in an additional aliquot of the same solvent (0.5 mL). *t*-BuOH (25 μL) was added for chemical shift reference and deuterium quantitation. A standard ^2H NMR spectrum (61.4 MHz) was first obtained at room temperature with sweep width 1645 Hz, 4 K data points zero-filled to 8 K, 90° pulse width, 1.245-s acquisition time, 48 287 scans. A second spectrum was obtained using the Bruker routine WATER.AUR, an inversion recovery water suppression sequence [P1 90°, P2 240°, D1 0.05 s, D2 0.2 s (to approximate T_1 of HOD), acquisition time 0.6226 s] with 102 200 scans accumulated: δ 1.28 (*t*-BuOH), 3.33 (H-5), 4.44 (H-3), and 4.92 (residual HOD). A third spectrum was obtained using the inversion recovery sequence at 330 K: δ 1.28, 3.34 (H-5), 4.42 (H-3), 4.51 (residual HOD inverted).

The combined IA (**1a**) fractions were similarly desalted, and center fractions containing pure **1a** were lyophilized and combined to give 54 mg, which was similarly analyzed by ^2H NMR.

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Comparison of Multicyclic Polyketides by Folding Analysis: A Novel Approach To Recognize Biosynthetic and/or Evolutionary Interrelationships of the Natural Products or Intermediates and Its Exemplification on Hepta-, Octa-, and Decaketides

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A folding code—using an analogous E/Z-terminology as known for the description of the stereoisomers of alkenes—is suggested as a helpful tool for the comparison of multicyclic polyketides and is applied in this paper to selected subgroups, such as tetracyclic decaketides as well as to tricyclic hepta- and octaketides. The result is that in contrast to structural similarities, a biosynthesis relationship regarding the polyketide synthases (PKS) can be proposed between the tetracyclines, the anthracyclines, and the angucyclines, while the biosynthesis of the tetracenomycins and the aureolic acid antibiotics must be performed via a differently operating polyketide synthase. This also leads consequently to a new biosynthetic hypothesis including the same bicyclic intermediate for the tetracyclines, anthracyclines and angucyclines. A biosynthetic relationship between the tetracenomycins and the aureolic acid antibiotics can be deduced from the folding analysis of these two antibiotic groups leading to a novel biosynthesis hypothesis for the latter group. In addition, the application of the folding code system to hepta- and octaketides shows that this concept can be used in general as a simple, but predictive approach to visualize biosynthetic interrelationships of multicyclic polyketides including their putative biosynthetic intermediates.

Biosynthetic studies, initiated by Cane and Hutchinson, have shown relationships between the polyketide synthases (PKS) of macrocyclic and polyether polyketides and fatty acid synthases.[1-4] The investigations have proven that

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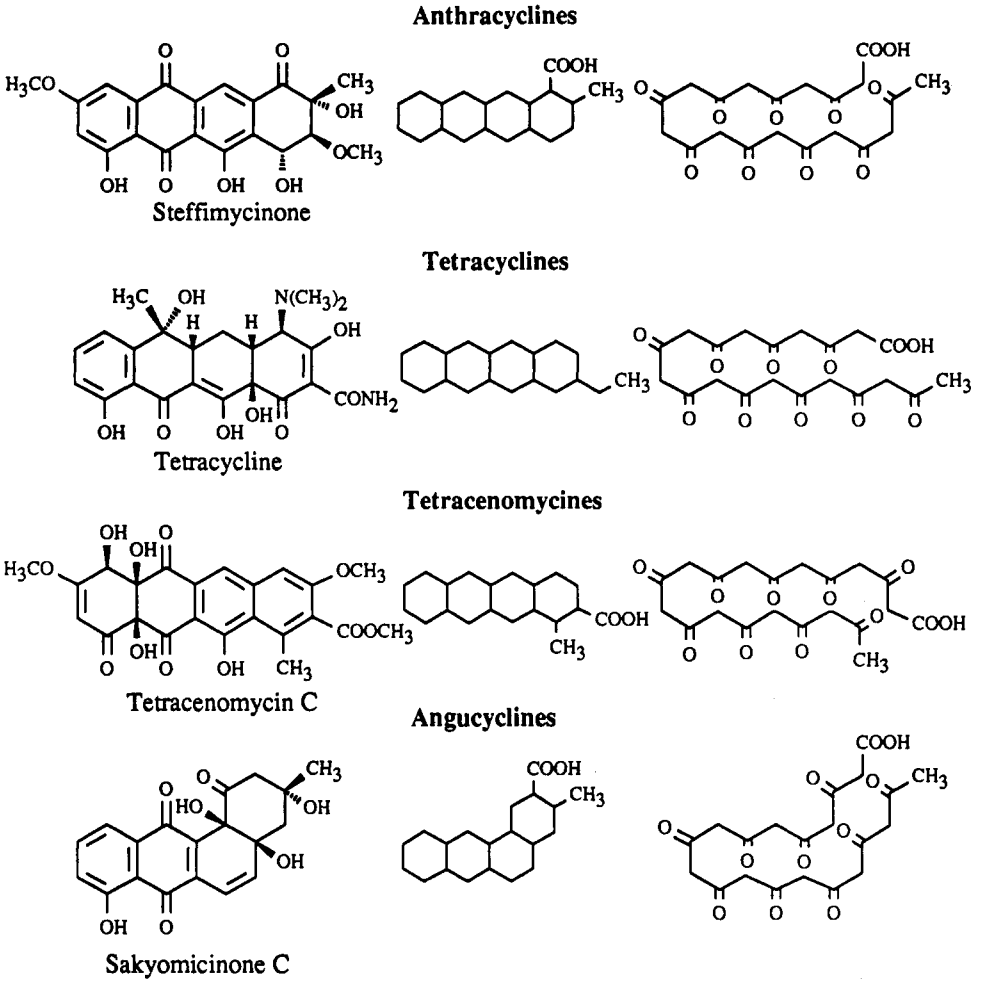

Figure 1. Hypothetical decaketide, simplified resulting tetracyclic frame, and one example of each of the four types of tetracyclic decaketide antibiotic groups.

a similar reduction cycle is used for both biosynthetic pathways with the difference that the reduction cycle[4,5] after each condensation of a C_2-unit is often incomplete in the case of the polyketide synthases (PKS). Differences regarding the stereochemical course of the reduction reactions were pointed out by Vederas et al.[5-7] This experimental work has been complemented theoretically, including the proposal of an evolutionary interrelationships between the polyketide synthases of polyether and macrolide antibiotics on the basis of structural and especially stereochemical similarities.[8,9]

For the aromatic, multicyclic polyketides a different type of PKS, namely a type-II PKS,[9e,f] catalyzes the intramolecular aldol reactions of the ring formations, rather than the one involved in macrolide and polyether assembly (type-I PKS). This requires an (at least almost) unmodified polyketide chain. These cyclase-catalyzed or spontaneous reactions[10] were supposed to happen more or less synchronously, thus establishing the characteristic multicyclic ring frame of a certain type of metabolite class. This dogma was weakened after McCormick et al. proposed tricyclic blocked mutant products of a tetracycline producer as shunt products of a hypothetical tricyclic intermediate[11,12] and had to be given up finally when Eckardt, Wagner et al. could isolate aklanonic acid, a tricyclic blocked mutant product, which could be proven to be an intermediate of the anthracycline biosynthesis.[13-16] Now, a subsequent stepwise ring closure mechanism is discussed to be more likely,[17] a theory which is also supported by the

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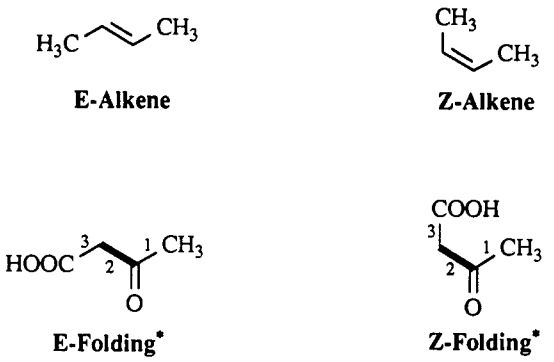

Figure 2. Folding terminology (example of a diketide, in analogy to the alkenes). * Of the highlighted bond (2) of a diketide relative to the bonds 1 and 3.

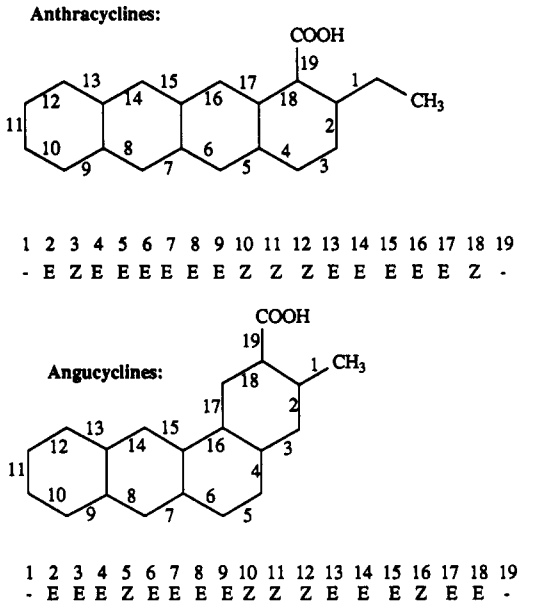

Figure 3. Numbering of the bonds and resulting folding code for the anthracyclines and the angucyclines.

findings of a tricyclic intermediate of the tetracenomycin biosynthesis,[18] as well as of the shunt products, which derive from a monocyclic and a bicyclic intermediate, respectively, in context with the actinorhodin biosynthesis (see also below).[19] All of these results could only be obtained after genetic manipulations of the parent microorganism strains.

Folding Code for Multicyclic Polyketides. Biosynthetic interrelationships of the multicyclic polyketides should be based on relationships of their polyketide synthases. This enzyme complex catalyzes the Claisen condensations of the monomeric ketide precursors as well as the folding and ring cyclizations (aldol reactions) into the final multicyclic frame, i.e., complex reaction sequences, among which the folding of the nascent polyketide chain is the most characteristic of the differently operating polyketide synthases. For the indirect comparison of the foldings, the following "folding code" terminology is suggested.[20] Here it is exemplified mainly on the analysis

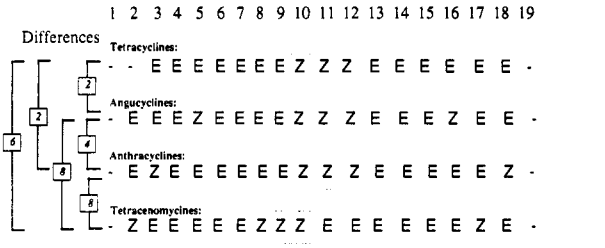

Figure 4. Folding codes of four types of tetracyclic decaketides in comparison; the boxes mark the "return" of the decaketide chain.

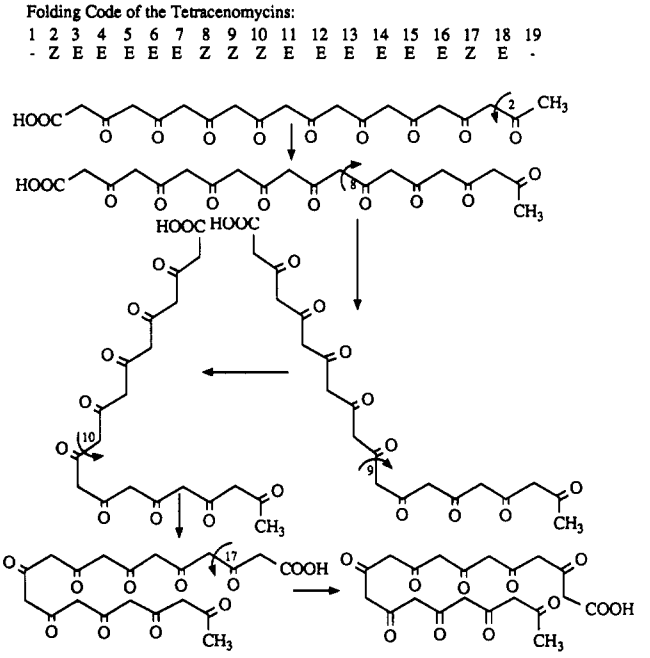

Figure 5. Establishment of the tetracenomycin folding from a hypothetical all-E decaketide with the folding code by flipping each of the "Z" bonds.

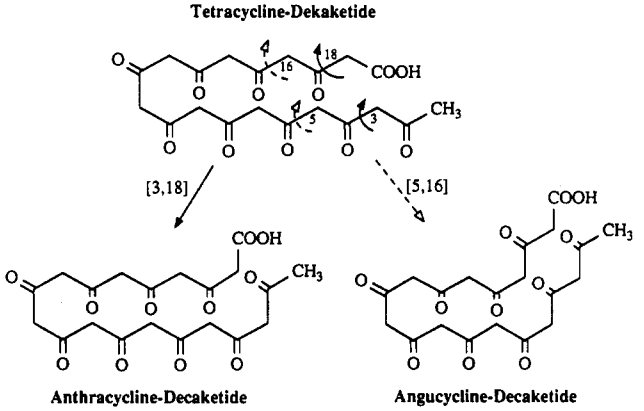

Figure 6. Interchange of the decaketides of tetracyclines, anthracyclines, and angucyclines by flipping of all bonds with different folding (see Figure 4).

of the folding of the hypothetical decaketide in the polyketide synthases of four different tetracyclic decaketide antibiotic groups, namely the tetracyclines, anthracyclines, angucyclines,[21] and tetracenomycins (Figure 1 shows the hypothetical decaketide, the resulting tetracyclic frame type, and for each group one structural example of the four mentioned antibiotic groups). The folding code can be established by (1) numbering the bonds of the hypothetical

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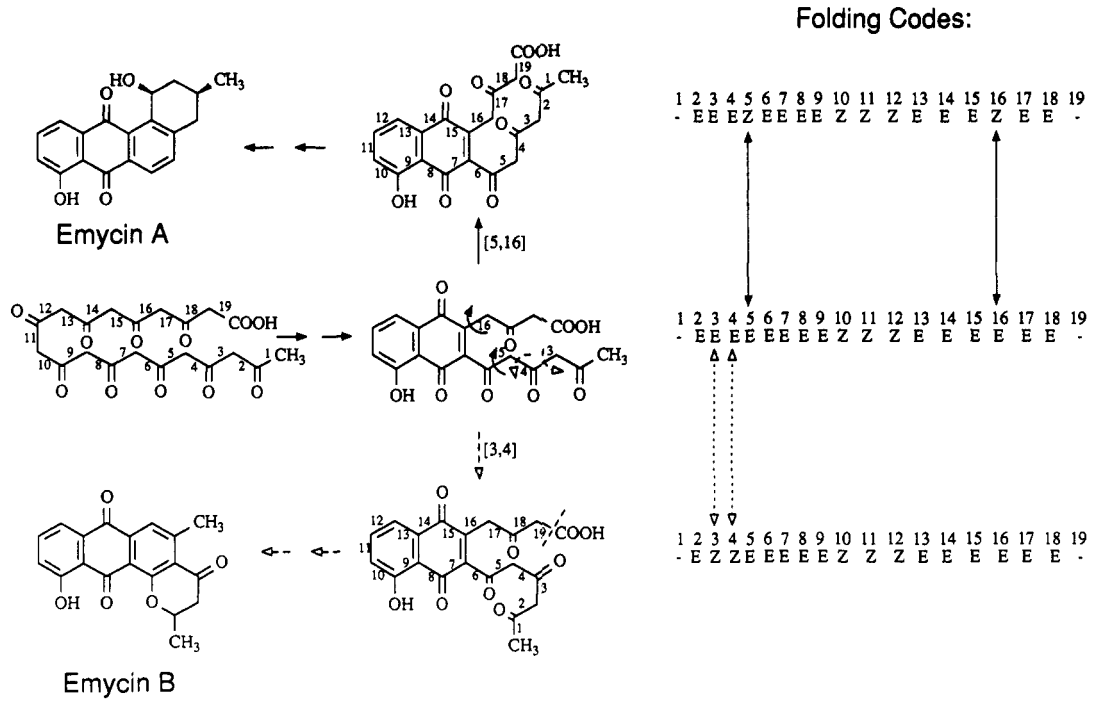

Figure 7. Comparison of the structures of the minor congener emycin B (ca. 3% of the production) and of the main product, the angucyclinone emycin A, with the folding code method reveals that both structures can be tracked back on a hypothetical common bicyclic biosynthetic intermediate.

polyketide or of the resulting frame (omitting some which are part of a longer ketide starter unit; e.g., in the case of a propionate-starter, the first bond has to be omitted for reasons of comparison with analogues having an acetate–starter, see also below) in a means of the growing polyketide chain, and (2) coding the C–C bonds which are part of the resulting multicyclic frame with the E/Z terminology (in analogy to the known E/Z system for the description of alkene stereoisomers, Figure 2).

Thus, the resulting folding code follows as an E/Z-sequence, as exemplified in Figure 3 for two of the four mentioned types of tetracyclic decaketide types. Figure 4 shows the folding codes of four tetracyclic decaketide types in comparison.

That the folding code really represents the shape of a characteristic four-ring frame can be proven by establishing the ring skeleton from a hypothetical (thermodynamically favored) all-*E*-decaketide by flipping each bond labeled with a "Z" (Figure 5 shows the example for the tetracenomycin type).

Comparison of the Folding Code and Consequences for Decaketides. The differences of the folding codes of the four types of decaketides relative to each other are also listed in Figure 4. A relationship between the tetracyclines, the anthracyclines, and the angucyclines can be proposed, because there are only two or four differences between any one of the folding codes, or in other words: only two isomerizations are necessary to change the tetracycline polyketide into the anthracycline or angucycline type, and four to interchange the anthracycline decaketide into the angucycline type (Figure 6). In this context it should be mentioned that Thomas already postulated a relationship between the tetracyclines and anthracyclines.[22] It is also remarkable that Gould and co-workers recently found a novel biosynthesis of an angucyclinone, whose acetate incorporation pattern could be explained by the postulation of an anthracyclinone-type intermediate.[23] The tetracenomycins have have more biosynthetic differences than one could expect from the structures. This is caused by the different "return-position" of the decaketide in the polyketide synthase (at the fifth ketide unit for the tetracenomycins, and at the sixth for the others) which has been discussed to be initiated by the reduction of a certain keto group[19] and which finally results in the first ring. Thus, the biosynthesis of the tetracenomycins must be catalyzed in a different way by its PKS, while the PKS of the other three antibiotic groups work obviously in a more similar way (a common "return-position").

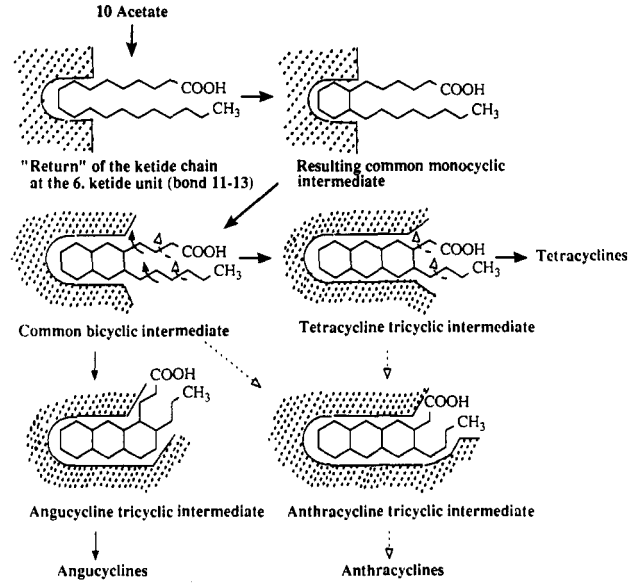

Figure 8. Hypothetical biosynthetic pathway of the tetracyclines, anthracyclines, and angucyclines: successive cyclizations and common mono- and bicyclic intermediates.

Thus, a biosynthesis hypothesis can be proposed, in which tetracyclines, anthracyclines, and angucyclines are

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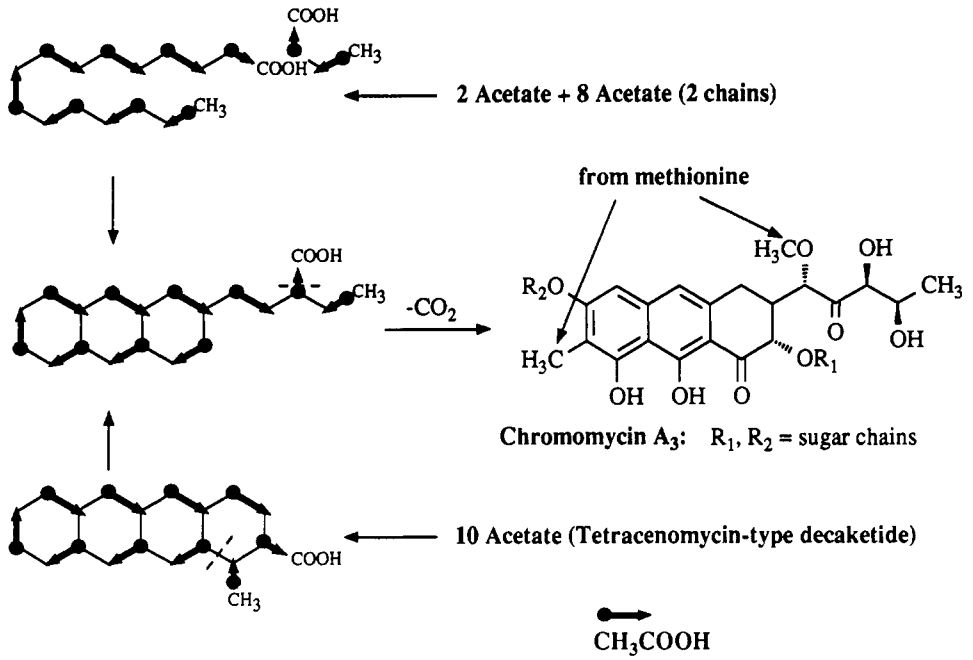

Figure 9. Biogenesis of chromomycin A_3: one of the discussed two-chain hypotheses[25] (top); the folding analysis reveals an alternative biosynthesis hypothesis via a single decaketide of the tetracenomycin type (bottom).

biosynthesized in a common pathway up to a bicyclic intermediate, from which the angucyclines branch off. The formation of emycin B, a small side product of the angucyclinone emycin A,[24] can be explained, if one assumes both antibiotics to derive from a putative bicyclic intermediate (Figure 7). Thus, the existence of the side product emycin B can be taken as evidence for a bicyclic intermediate of the emycin A biosynthesis. The anthracyclines branch off either also at the stage of a bicyclic intermediate or even later at the stage of a tricyclic intermediate (Figure 8).

The folding type represented by tetracenomycins seems to be more rare in nature. But recently, the first biogenetic studies of an aureolic acid metabolite, chromomycin A_3, were published.[25] The authors discussed different pathways with at least two independent polyketide chains. As a consequence of the folding code analysis an obvious relation to the tetracenomycins can be deduced, since only a scission of one bond of a "tetracenomycin" intermediate is necessary to obtain the acetate incorporation pattern found for chromomycin A_3 (Figure 9).

Application of the Folding Code to Tricyclic Hepta- and Octaketides. To show that the folding code concept is not only limited to tetracyclic decaketides, it also is applied here to other groups of multicyclic polyketides, namely to the tricyclic hepta- and octaketides, including those of which biosynthetic interrelations are well studied and proven by genetic methods.[19,26]

The three tricyclic heptaketides altenariol,[27] deoxyherquienone,[28] and griseofulvin[29] seem structurally unrelated to each other. However, the application of the folding code indicates that a common monocyclic intermediate and thus a biosynthetic or evolutionary relationship might be possible (Figure 10).

As another example, the comparison of actinorhodin with its biosynthetically related shunt products aloesaponarin II and mutactin can be visualized with the folding code (Figure 11, the identical "return position" at the fourth ketide unit, i.e., "Z"-bonds 6, 7, and 8, is an indication of a common monocyclic intermediate).[19,26] Since it is a fact that all three metabolites stem from the same organisms and that the same PKS has been blocked in different positions, they all arise from a different folding pattern of the same polyketide, and moreover, they all have a common monocyclic intermediate. In contrast, a biosynthetic relationship of actinorhodin with other octaketides like islandicin[30] or eleutherinol[31] or between those latter two can be excluded ("return" at bonds 7–8–9 and 8–9–10, respectively, see Figure 11).

Conclusions

The folding code is a simple, practical approach to compare some aspects of the properties of different polyketide synthases, after the biogenetic origin of a multicyclic molecular backbone has been proven to derive from the polyketide pathway and after the direction of the polyketide chain has been resolved unambiguously. It offers a novel approach to visualizing relationships between putative polyketide intermediates as well as cyclized, aromatic products and thus may one help to see nonobvious precursor–product relationships. A possible relationship is already indicated when the first return (in the folding code a triple "Z") of a polyketide chain appears at the same position, i.e., a possible common monocyclic intermediate. Further similarities in the folding code mean a closer relationship.

The results from comparison of the tetracyclic decaketides are in some contrast to chemical structure similarities and other discussed hypotheses,[26] and thus add some new viewpoints, such as the described biosynthetic

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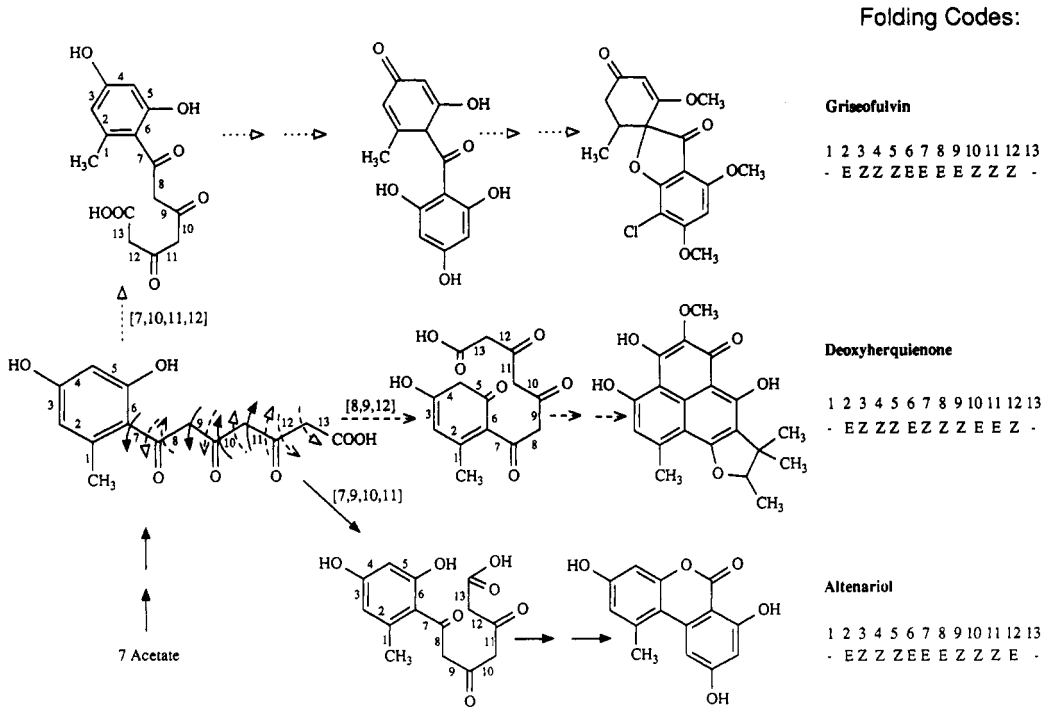

Figure 10. Folding codes and possible relationship between three tricyclic heptaketides: A hypothetical common monocyclic intermediate of all three biosyntheses?

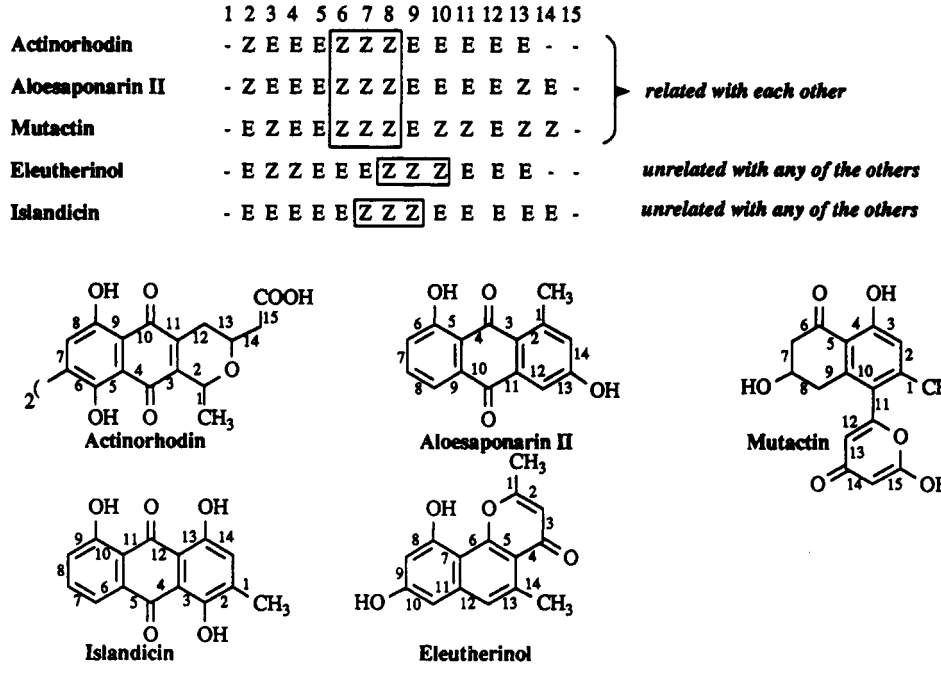

Figure 11. Application and results of the folding code concept to five different octaketides. The proven relationship[19,26] of actinorhodin, aloesaponarin II, and mutactin can also be visualized with their folding code similarities.

relationship hypotheses (Figures 7–9). Because of the structural similarities one could assume a stronger biosynthetic relationship between tetracyclines, anthracyclines, and tetracenomycins, since all three of them possess linearly assembled tetracyclic ring frames, while the angucyclines seem to be different due to their benz[*a*]-anthracene frame.[26] The folding code analysis has pointed to a similarity of the biosyntheses of tetracyclines, anthracyclines, and angucyclines at the stage of the polyketides synthases despite their structural differences. The genes encoding the polyketide synthases of the anthracyclines and angucyclines have apparently remained more closely associated with the polyketide synthase system of the tetracyclines through the process of evolution, while the tetracenomycins are more likely biosynthesized via an evolutionally separately developed and functioning polyketide synthase.

The folding code method, however, is limited to aromatic, multicyclic polyketides, i.e., to those whose biosynthesis is catalyzed by a type-II PKS, and which thus derive from an (at least nearly) unreduced polyketide chain. The application of the folding code requires the study of the biogenesis and is per se limited on those molecules whose biosyntheses do not include any rearrangement. If a rearrangement can be tracked back on a certain intermediate, the latter one can be analyzed. Thus,

in context with the biosynthetic studies on the kinamycins[32] and on metabolites of the toromycin/gilvocarcin group (e.g., chrysomycin B[33]) an angucyclinone-type intermediate was proven and assumed, respectively, which undergoes a rearrangement leading to the found structures. Thus, a strong similarity of the polyketide synthases of these antibiotic groups with the angucycline-PKS is or seems to be evident.

Such similarities of the polyketides synthases may have implications in future biosynthetic studies on the different types of multicyclic polyketides which cannot be carried out without the tools of genetic engineering and/or mutagenesis. This also justifies further biosynthetic studies on the angucyclines, which will be focused on in early biosynthetic steps, since these may be also relevant for the clinically important tetracyclines and anthracyclines.

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The Synthesis and Reactivity of [N(8)–C(3′)]-Cyclized Bicyclomycin. Evidence of the Role of the C(1)-Triol Group in Bicyclomycin-Mediated Processes

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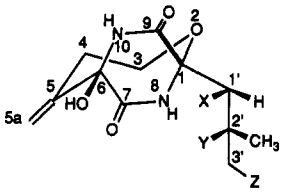
The C(1) triol group in the antibiotic, bicyclomycin (1) has been proposed to play an integral role in the bonding of key protein nucleophiles to the distal C(5)–C(5a) terminal double bond in the drug. Evidence in support of this concept has been provided by the comparison of the reactivities of bicyclomycin (1), the [N(8)–C(3′)]-cyclized bicyclomycin adduct 3, 2′,3′-bicyclomycin acetonide (17), and the acetonide derivative of 3, 18, with sodium ethanethiolate. Significantly, 3 displayed enhanced reactivity versus 1, 17, and 18 in this transformation. The controlling factors for the increased reactivity of 3 have been discerned and the importance of the C(1′) hydroxyl group delineated. Key kinetic parameters are reported for the treatment of both 3 and 17 with 2-mercaptopyridine. Structural details are provided for both C(5a) thiolate and amine adducts of 3. The importance of these findings in relation to the mode of action of bicyclomycin are briefly discussed.

Bicyclomycin (1) is a structurally unique antibiotic possessing a diverse spectrum of biological activity.[1-4] Important architectural features in 1 include the bicyclic [4.2.2] ring structure, the C(5)–C(5a) exomethylene group, and the C(1) triol moiety. Most mechanistic proposals concerning the mode of action of 1 suggest that nucleophilic residues present in key proteins involved in bacterial cell wall growth irreversibly bind to the terminal double bond at C(5).[5-8] The role of the appended C(1) triol group in these transformations is unclear. This information remains an important objective in the elucidation of the biological pathway of this commercial antibiotic.

Several studies pertinent to this issue have appeared. First, all structural modifications of the C(1) triol moiety in bicyclomycin led to a pronounced reduction in the biological activity of the drug candidates.[6,9,10] Second,



1 X = Y = Z = OH
4 X = Y = OH; Z = $OS(O)_2CH_3$
14 X = OH; Y, Z = O
15 X = Y = OH; Z = SCH_2CH_3
16 X = Y = OH; Z = $OC(O)NHCH_2CH_3$
17 X = OH; Y, Z = $OC(CH_3)_2O$

Williams and co-workers reported that thiolate addition to the bicyclomycin mimic 2 at "pH" 12.5 in tetrahydrofuran (THF)–water (3:1) mixtures was promoted by intramolecular transfer of a proton from the C(1′) hydroxyl group to the C(9) carbonyl moiety.[10] Third, Kohn and Abuzar demonstrated that modification of the C(1) triol moiety in 1 both impeded the functionalization of the exomethylene group and prevented the formation of bicyclomycin-derived piperidinedione-type adducts at near neutral "pH" values.[8d] In this paper, we report that reaction at the exomethylene group in the annelated bicyclomycin adduct 3[9,11] with thiolate species proceeded *more rapidly* than the corresponding process with 1. Analysis of the structural factors responsible for the enhanced reactivity of 3 versus 1 provides evidence for the

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(11) The following uninverted Chemical Abstracts Index name for 3 modified by current IUPAC guidelines has been kindly provided by Dr. P. M. Giles (Chemical Abstract Services): (5*R*,9*R*,10*S*,10a*S*)-hexahydro-5,9,10-trihydroxy-9-methyl-4-methylene-8*H*-5,10a-(iminomethano)-6*H*-pyrrolo[2,1-*b*][1,3]oxazocine-6,11-dione.

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