Biosynthesis of Capreomycin. 1. Incorporation of Arginine

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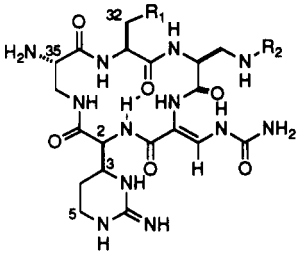
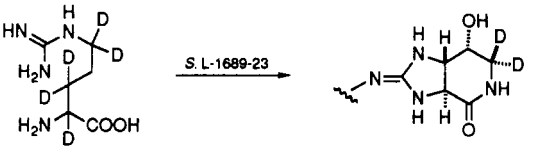
The biosynthesis of the capreomycidine moiety of the capreomycin antitubercular antibiotics has been studied in *Streptomyces capreolus* A250. Initial experiments with [1-^{14}C]arginine confirmed that this is the primary precursor. Subsequent incorporation of [2,3,3,5,5-$^{2}H_5$]arginine afforded samples of capreomycins IA (**1a**) and IB (**2a**) that were analyzed by ^{2}H NMR spectroscopy. Deuterium labels had been retained from H-3 and H-5 of the arginine, but not from H-2, which supports the involvement of an α,β-dehydroarginyl intermediate, presumably formed as part of a peptide.

Capreomycin, a metabolite of *Streptomyces capreolus*, was first described in 1960.[1] It was subsequently reported to be composed of two components (I and II) in 1962,[2] and in 1965 was finally shown to actually contain the four components IA, IB, IIA, and IIB (**1**, **2**, **3**, and **4**, respectively).[3] The general properties of the capreomycins were recognized as being related to those of the strongly basic, cyclic peptide viomycin,[4] a potent tuberculostatic antibiotic.[5-7] A structure was proposed for capreomycin,[8] but was later revised.[9,10] L-Capreomycidine, **5**, the most unusual amino acid component, has been synthesized,[11] and total syntheses of capreomycins IA and IB have also been reported.[12] Additional members of this group, now called the tuberactinomycins, have subsequently been characterized from other species.[13-16]



1: R_1 = OH, R_2 = β-Lysine
2: R_1 = H, R_2 = β-Lysine
3: R_1 = OH, R_2 = H
4: R_1 = H, R_2 = H

Scheme I



8a **7a**

Capreomycin is active against a number of Gram-positive and Gram-negative bacteria, but is primarily effective against mycobacteria. It has been used in the treatment of strains of *Mycobacterium tuberculosis* that are not responsive to standard antitubercular drugs such as streptomycin or rifampicin.[17,18] The chemical and biological properties of capreomycin and the other tuberactinomycins have been reviewed.[19,20]

Our interest in capreomycin derived from the presence of the L-capreomycidine moiety, which was proposed[21] to be an intermediate in the biosynthesis of streptolidine, **6**,[22] a key component of the streptothricin antibiotics,[23,24] as represented by streptothricin F, **7** (Figure 1).[25,26]

We have studied the biosynthesis of **7** extensively,[27-32] and the intermediacy of **5** was fully consistent with results from the incorporation of a series of ^{15}N-^{13}C-labeled arginines.[27,29] In further probing the mechanism of formation of the cyclic guanidine ring of the putative capreomycidine moiety, however, unusual results were obtained with a variety of arginines labeled with deuterium at C-2 and/or

(1) Herr, E. B., Jr.; Haney, M. E.; Pittenger, G. E.; Higgens, C. E. *Proc. Ind. Acad. Sci.* **1960**, *69*, 134.
(2) Stark, W. M.; Higgens, C. E.; Wolfe, R. N.; Hoehn, M. M.; McGuire, J. M. *Antimicrob. Agents Chemother.* **1962**, 596-606.
(3) Stark, W. M.; Boeck, L. D. *Antimicrob. Agents Chemother.* **1965**, 157-164.
(4) Bycroft, B. W.; Cameron, D.; Hassanali-Walji, A.; Johnson, A. W.; Webb, T. *Experientia* **1971**, *27*, 501-503.
(5) Bycroft, B. W. *J. Chem. Soc., Chem. Commun.* **1972**, 660-661.
(6) Bycroft, B. W. *Proc. Am. Pep. Symp.* **1972**, 665-670.
(7) Bycroft, B. W.; Cameron, D.; Croft, L. R.; Hassanali-Walji, A.; Johnson, A. W.; Webb, T. *Nature* **1971**, *231*, 301-302.
(8) Shiba, T.; Nomoto, S.; Wakamiya, T. *Experientia* **1976**, *32*, 1109-1111.
(9) Nomoto, S.; Teshima, T.; Wakamiya, T.; Shiba, T. *J. Antibiot.* **1977**, *30*, 955-959.
(10) Shiba, T.; Ukita, T.; Mizuno, K.; Teshima, T.; Wakamiya, T. *Tetrahedron Lett.* **1977**, 2681-2684.
(11) Shiba, T.; Nomoto, S.; Teshima, T.; Wakamiya, T. *Tetrahedron Lett.* **1976**, 3907-3910.
(12) Wakamiya, T.; Shiba, T.; Kaneko, T. *Tetrahedron Lett.* **1970**, 3497-3500.
(13) Nagata, A.; Ando, T.; Izumi, R.; Sakakibara, H.; Take, T.; Hazano, K.; Abe, J. *J. Antibiot.* **1968**, *21*, 681-687.
(14) Izumi, R.; Noda, T.; Ando, A.; Teruo, T.; Nagata, A. *J. Antibiot.* **1972**, *15*, 201-207.
(15) Ando, T.; Matsuura, K.; Izumi, R.; Noda, T.; Take, T.; Nagata, A.; Abe, J. *J. Antibiot.* **1971**, *24*, 680-686.
(16) McGahren, W. J.; Morton, G. O.; Kunstamann, M. P.; Ellestad, G. A. *J. Org. Chem.* **1977**, *42*, 1282-1286.
(17) Martindale, W. *The Extra Pharmacopoeia*; Blacow, N. W., Ed.; Hazell Watson and Viney Ltd.: Great Britain, 1972; pp 1585-1586.
(18) Herr, E. B., Jr.; Redstone, M. O. *Annal. N. Y. Acad. Sci.* **1966**, *135*, 940-946.
(19) Herr, E. B., Jr.; *Antimicrob. Agents and Chemother.* **1962**, 201-212.
(20) Shiba, T. *Bioactive Peptides Produced by Microorganisms*; Umezawa, H., Takita, T., Shiba, T., Eds.; Halsted Press: New York, 1978; pp 71-86.
(21) Bycroft, B. W. *Nature* **1969**, *224*, 595-597.
(22) Bycroft, B. W.; King, T. J. *J. Chem. Soc., Chem. Commun.* **1972**, 652-654.
(23) Khoklov, A. S. *J. Chromatogr. Libr.* **1978**, *15*, 617-713.
(24) Borders, D. B. *Methods in Enzymology*; Hash, J. H., Ed.; Academic Press: New York, 1975; pp 256-263.
(25) Waksman, S. A.; Woodruff, H. B. *Proc. Soc. Exp. Biol. Med.* **1942**, *49*, 207.
(26) Van Tamelen, E. E.; Dyer, J. R.; Whalen, H. A.; Carter, H. E.; Whitfield, G. B., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 4295-4296.
(27) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 2871-2874.
(28) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 4639-4640.
(29) Martinkus, K. J.; Tann, C.-H.; Gould, S. J. *Tetrahedron* **1983**, *39*, 3493-3505.
(30) Thiruvengadam, T. K.; Gould, S. J.; Aberhart, D. J.; Lin, H.-J. *J. Am. Chem. Soc.* **1983**, *105*, 5470-5476.
(31) Paliniswamy, V. A.; Gould, S. J. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2283-2286.
(32) Gould, S. J.; Lee, J.; Wityak, J. *Bioorg. Chem.* **1991**, *19*, 333-350.

0022-3263/92/1957-5214$03.00/0

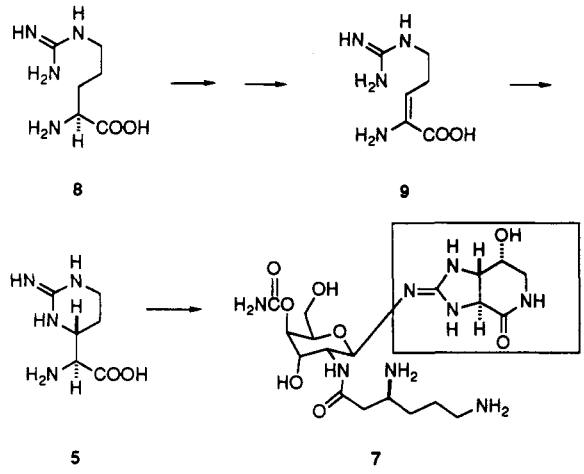


Figure 1. Proposed biogenesis of streptolidine moiety, 6 [shown in box], of the antibiotic streptothricin F, 7.

C-3.[29] These efforts culminated with the result shown in Scheme I for the incorporation of the pentadeuteriated arginine **8a**,[32] which was inconsistent with the original proposal involving α,β-dehydroarginine,[21] **9**, but could still be rationalized by a pathway via 3-hydroxy- and 3-keto-arginine.[29] However, when both *erythro*- and *threo*-3-hydroxyarginines were synthesized and tested as intermediates the results were negative;[32] neither was incorporated nor apparently produced de novo. We, therefore, decided to directly investigate the biosynthesis of **5** in *S. capreolus*.

Results and Discussion

Fermentation of *S. capreolus* in a variety of media have been reported,[2] including two defined media that purportedly produced mainly IA or IB.[3] However, we found that in each case both antibiotics were produced in roughly equal amounts, as observed by TLC. We therefore chose to use the complex medium, which produced the highest titers. In this case, bioassay with *Bacillus subtilis* ATCC 6633 was sufficiently sensitive for our purposes (MIC 3.13 μg/mL), and much simpler than the previously reported[2] turbidometric assay. Typically, production started 1 day after inoculation of the production broth and reached the maximum titer after 6 days.

An efficient purification of IA and IB was established using commercial antibiotic and required some modification of the literature protocol.[8,9] Initial attempts using Amberlite CG-50 (NH_4^+) ion-exchange resin with a gradient of ammonium acetate (0.4–0.8 M, pH 9.0) were disappointing, requiring 5 days and yet still not giving complete separation. Attempts with CM-Sephadex (0–0.3 M NH_4OAc gradient) gave no significant separation; neither did chromatography on Sephadex LH-20. However, we fortuitously discovered that the CG-50 chromatography worked much better and more rapidly at 4°, and an almost base-line separation was achieved in 2 days with a 0.4–0.8 M NH_4OAc gradient followed by isocratic elution at 0.8 M. Attempts to carry out the initial ion-exchange separation using volatile buffers (ammonium carbonate or triethylammonium carbonate) failed to give any significant separation. The literature desalting, which used a CG-50 (pyridinium form) ion-exchange resin eluted with acetic acid, followed by neutralization with pyridine, was replaced with size-exclusion chromatography using Sephadex G-10 eluted with water. In this manner, each capreomycin could normally be desalted in a matter of hours, although care was needed to avoid the last antibiotic fractions since they were contaminated with ammonium acetate. Fractions were monitored at 270 nm and lyophilized. Appropriate fractions containing IA or IB were then combined, dissolved in a minimum volume of water, and recrystallized by addition of ethanol. With this part of the new protocol established, crude fermentation mixtures were easily brought to the required degree of purity for the ion-exchange chromatography using the initial steps from the literature.[33]

For the biosynthetic experiments, fermentations were carried out with 200 mL of production broth in a 1-L Erlenmeyer flask. This provided ca. 450 mg of capreomycin equivalents in the crude broth, and after purification, ca. 150 mg each of IA and IB were obtained. On the basis of the time-course of the fermentation, pulse feedings were then carried out by adding one-third of the precursor at 12, 32, and 56 h after inoculation of the production broths, and the fermentations were continued a total of 6 days.

Initially, 13.76 μCi of DL-[1-^{14}C]arginine was fed and 580 mg of capreomycins was produced (bioassay). Ninety-two milligrams of pure IA were obtained and recrystallized to constant specific activity (6.5×10^3 dpm/mg), yielding a 6.25% incorporation, which corresponded to a 12.5% incorporation if both capreomycins had the same specific activity. Unfortunately, in this instance a salt-free sample of IB could not be obtained. In a second experiment, L-[U-^{14}C]arginine was fed, 517 mg of the capreomycins was produced, and samples of IA (1.11×10^4 dpm/mg) and IB (1.65×10^4 dpm/mg) were obtained. These correspond to incorporations of 8.5% for IA and 12.7% for IB. Since the combined level of incorporation in the second experiment was roughly twice that of the first, it suggests that only L-arginine is biologically useful in this pathway, as was the case for streptothricin F.[28]

We next fed 60 mg of unlabeled arginine to a new broth using the same protocol. In this case, 526 mg of total capreomycins was obtained, indicating that it was possible to feed significant quantities of arginine without adversely affecting the capreomycin production. This allowed us to next feed a sample of the deuteriated arginine **8a** (60.1 mg), mixed with 11.42 μCi of DL-[1-^{14}C]arginine. As before,[32] the deuterium labels at H-5 were to provide an internal reference for the presence and relative amount of deuterium at those positions derived from H-2 and H-3. This time 972 mg of the capreomycins were produced. The workup was monitored by radioactivity and indicated the fermentation had proceeded unexceptionally.

The samples of capreomycins IA and IB, **1a** and **2a**, respectively, derived from **8a** were each lyophilized from deuterium-depleted water and then analyzed by ^{2}H NMR spectroscopy in the same solvent. The full proton assignments had been previously obtained from a series of 1D and 2D NMR experiments.[34] Thus, the expected resonances of interest for each compound would be at δ 5.05 (H-2), 4.40 (H-3), and 3.34 (H-5). Deuterium-depleted water was used to minimize the normal solvent peak, which nearly overlapped with the H-2 resonance. 2-Methyl-2-propanol was included as a chemical shift reference and to quantitate the deuterium enrichment. The spectrum of **2a** showed a clear resonance at δ 3.34 for the H-5 hyrogens, as expected. A smaller broad hump at δ 4.40, partially obscured by the water peak, suggested the presence of deuterium at H-3, as well. The smooth curve of the downfield side of the water peak suggested that the

(33) Herr, E. B., Jr.; Hamill, R. L.; McGuire, J. M. Eli Lilly and Co. British Patent 920,563, Mar 6, 1963, Appl. Aug 7, 1961; *Chem. Abstr.* **1964**, *60*, 1078e.
(34) Minott, D.; Gould, S. J. Unpublished results.

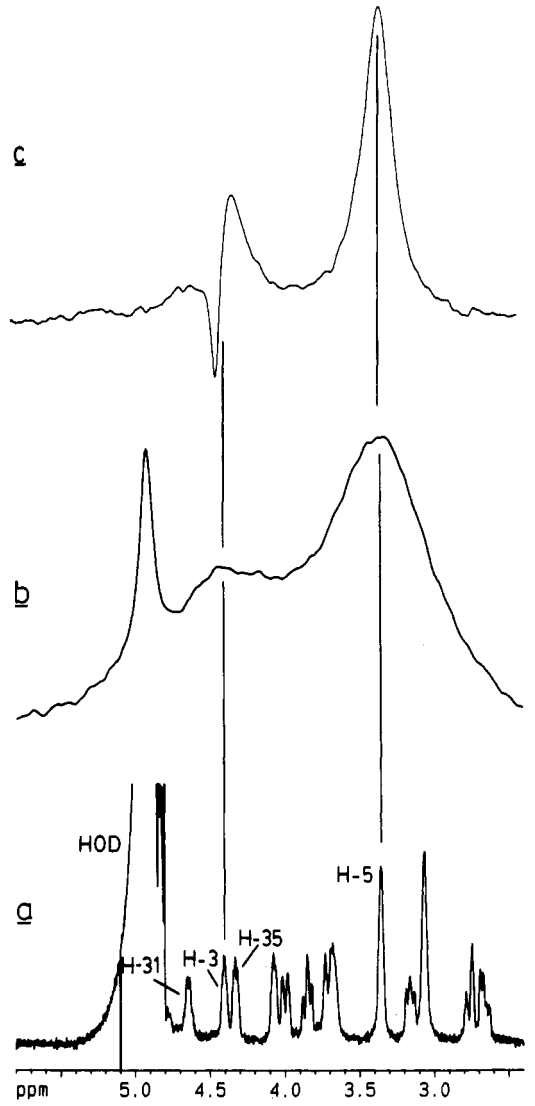
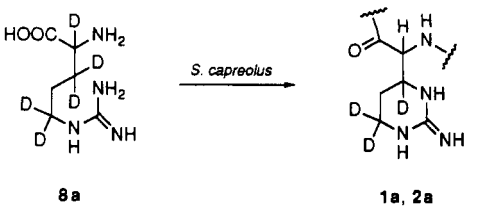


Figure 2. Partial NMR Spectra of **2a**. (a) 400-MHz ^1H NMR spectrum in D_2O. (b) 61.4-MHz ^2H NMR spectrum in ^2H-depleted H_2O at room temperature, showing the presence of deuterium enrichment at H-3 and H-5. (c) As in b but at 330 K showing an absence of deuterium enrichment at H-2; residual HOD appears inverted at δ 4.5 due to inversion recovery water suppression pulse.

H-2 label of arginine **8a** had been lost. To confirm the presence of deuterium at H-3, but not at H-2, the FID was acquired again at room temperature and at 330 K, now including a presaturation pulse to diminish the residual water resonance. The results, shown in Figure 2b and 2c, respectively, clearly reveal the deuterium at H-3 but, again, there was no evidence of deuterium at H-2. In order to gain an approximate value for the deuterium enrichments, the curve of each resonance trace in the first spectrum was extended to the base line. In comparison with that from the 2-methyl-2-propanol resonance, these measurements gave deuterium contents of 2.5 and 1.4 μmol for H-5 and H-3, respectively, corresponding to enrichments of 3.2 and 1.7%. These are roughly in the 2:1 ratio expected for retention of both hydrogens at H-5 and one hydrogen at H-3 of the arginine fed. The ^2H NMR spectrum for **1a** was essentially the same.

During our initial fermentation studies we confirmed the previous observation[8,9] that IB appeared earlier in the fermentation than IA. Since they differ only in the absence or presence of a hydroxyl at C-32, it was possible that IA was derived by hydroxylation of IB, rather than a real substitution of a serine for an alanine in the biosynthesis.



8a **1a, 2a**

In an attempt to test this, a sample (25.3 mg, 0.155 μCi) of the IB obtained from the DL-[1-^{14}C]arginine feeding was now fed to a new fermentation of *S. capreolus*. Workup revealed the presence of IB but of very little IA. When the mixture was separated and the individual capreomycins analyzed for the presence of ^{14}C, the IB sample retained nearly all of the radioactivity that had been fed, while the small IA sample was essentially inactive. At this point it is not clear whether IA is produced independently from IB or that the IB fed could not enter the cell due to impermeability to the (potentially toxic) compound.

Since the manner of incorporation of **8a** into the capreomycins is apparently quite different than into streptothricin F, **7a**, the present study does not shed light on the latter process. However, the capreomycin results are readily explained by intramolecular addition of the guanidine to an α,β-dehydroarginyl residue. One might expect free dehydroarginine, which would have appeared to be necessary for **7**, to rapidly hydrolyze, being a primary enamine. However, since in the capreomycins capreomycidine is actually found as part of a peptide structure, and *N*-acyl-α,β-dehydroamino acids are stable, it is reasonable to assume that the capreomycidine ring arises from postassembly modification of arginine in a peptide intermediate. This and other details of capreomycin biosynthesis will be reported in due course.

Experimental Section

General Procedures. *S. capreolus* A250 was obtained from the Eli Lilly Co. *B. subtilis* ATCC 6633 was obtained from Becton Dickinson Microbiology Systems, and Bacto agar was obtained from Difco Laboratories.

Maintenance of *S. capreolus*. *S. capreolus* A250 was grown on the following agar: dextrin (10.0 g), yeast extract (1.0 g), N-Z amine A (2.0 g), beef extract (1.0 g), $CoCl_2 \cdot 6H_2O$ (0.01 g), granulated agar (20.0 g), and deionized water to 1 L, adjusted to pH 7.0 with NaOH prior to sterilization. Slants were incubated at 28 °C for 10 days, stored at 4 °C, and used for inoculation of broths. Agar in a petri plate was inoculated and incubated at 28 °C for 9 days, and agar plugs from the plate were preserved in liquid nitrogen for long-term storage.

Fermentation of *S. capreolus* A250. A sterile 0.1% Tween 80 solution (5.0 mL) was added to a 10-day old slant (15 mL) of *S. capreolus*, and the spores were suspended in the aqueous solution. A portion of the spore suspension (0.25 mL) was used to inoculate a seed broth (60 mL) containing tryptone (0.3 g), yeast extract (0.15 g), glucose (0.9 g), $MgSO_4 \cdot 7H_2O$ (0.18 g), $FeSO_4 \cdot 7H_2O$ (0.06 mg) (0.1 mL of a 0.6 g/L stock), $CaCl_2 \cdot 2H_2O$ (0.015 g) (1.0 mL of a 15 g/L stock), and deionized water to 60 mL, which had been adjusted to pH 7.5 with 2% KOH before sterilization.

The flask (250 mL) was incubated at 30 °C, 250 rpm for 42 h, and was used (5% v/v) to inoculate production broths (200 mL in 1-L Erlenmeyer flasks) containing glucose (5.0 g), Bactopeptone (3.0 g), N-Z amine A (0.8 g), blackstrap molasses (2.0 g), starch (2.0 g), $MgSO_4 \cdot 7H_2O$ (1.0 g), $CaCO_3$ (0.4 g), and hot tap water to 200 mL. The pH (6.5) was unadjusted.

Bioassay of Capreomycins IA and IB against *B. subtilis* ATCC 6633. A base agar layer (20 mL) was prepared (0.5% trypticase peptone + 1.5% Bacto agar), and a seed layer (6 mL), consisting of a diluted *B. subtilis* ATCC 6633 saline suspension (0.71 mL) in the same agar (32 mL), was added. Sample solutions (100 μL) of capreomycins IA and IB were applied to the plates in stainless steel cylinders. The plates were incubated overnight at 37 °C and the concentrations determined from a plot of zone

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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