SPECTOMYCINS, NEW ANTIBACTERIAL COMPOUNDS PRODUCED BY *Streptomyces spectabilis*: ISOLATION, STRUCTURES, AND BIOSYNTHESIS

ANDREW L. STALEY and KENNETH L. RINEHART*

Roger Adams Laboratory, University of Illinois,
600 So. Mathews Ave., Urbana, IL 61801, U.S.A.

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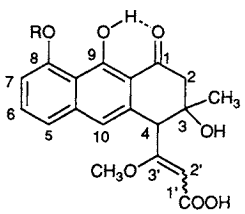
Three new tetrahydroanthracene antibiotics have been isolated from modified culture broths of *Streptomyces spectabilis*. The new compounds, spectomycins A1, A2 and B1, exist as monomeric ($C_{20}H_{20}O_7$, A1; $C_{19}H_{18}O_7$, A2) and as symmetrical dimeric ($C_{38}H_{34}O_{14}$; B1) forms. Only spectomycin B1 has moderate activity against Gram-positive microorganisms. We report here the structure elucidation and biosynthetic origin of these compounds.

During our ongoing studies of the biosynthesis of the streptovaricins,[1] it became necessary to develop a medium that was suitable for the production of mutants of *S. spectabilis*. We found an appropriate, soluble synthetic growth medium that supported high production and growth profiles of the organism, but bioautography of the fermentation extracts indicated that the bioactivity *vs B. subtilis* was not due to the production of any known streptovaricins. Instead, the activity was associated with two series of new, unidentified metabolites whose structures have been determined by spectral and chemical methods. We report here the structure elucidation of the first series of compounds, which we have trivially named spectomycins A1, A2, and B2 (**1a**~**c**).[2] These compounds, which proved to be members of the aureolic acid class of antibiotics, are most closely related to julimycins[3,4] and exist as a mixture of monomeric and dimeric species. The second series of bioactive metabolites (deoxymonamycins)[2] will be reported separately.

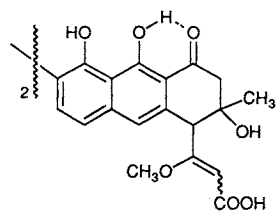
Results

Detection and Isolation

A number of synthetic and semisynthetic media were screened for the production of bioactive components by dipped disk assay *vs B. subtilis*. The filtered culture broth and an ethyl acetate extract of the broth (2.5-fold concentration of the organic extract versus the culture broth) were assayed separately. Antimicrobial activity was detected in most of the culture broths, but organic-extractable activity was



1a Spectomycin A1 (R=CH_3)
1b Spectomycin A2 (R=H)



1c Spectomycin B1

observed only in Medium I (1.0% glucose, 1.0% $(NH_4)_2SO_4$, 0.35% NaCl, 0.25% K_2HPO_4, 0.15% KH_2PO_4, 0.01% $CaCO_3$, and 0.01% $Na_2S \cdot 9H_2O$ in tap water) and Medium II (Medium I supplemented with 0.5% yeast extract (DIFCO)). Production of the organic-extractable activity commenced at 48 hours after inoculation and persisted until the culture was worked up at 140 hours after inoculation. Although good production of streptovaricins was noted by TLC of the extract from Medium II, none could be detected in the extract from Medium I. Bioautography of the extract from Medium I (silica gel, chloroform-methanol, 9:1) *vs B. subtilis* indicated a large zone of activity from Rf 0.4 to 0.6, but this activity could not be attributed to the production of any known streptovaricin components.

A large-scale fermentation was conducted in 200 liters of Medium I. The whole broth was extracted at the final pH 3.9 with ethyl acetate (3~4 liters per 12-liter batch of beer). The combined extracts were concentrated to 500 ml and partitioned with 5% aqueous sodium bicarbonate. The aqueous layers, after acidification and re-extraction, provided a mixture of crude carboxylic acids that was separated by chromatography on silica gel and Sephadex LH-20. Three main components were collected: **1a** (9.1 mg), **1b** (120 mg), and **1c** (324 mg). The dimeric compound **1c** showed an MIC of 2 μg/ml against *B. subtilis* by dipped disk assay, but was inactive against *E. coli*, *S. cerevisiae*, and *P. atrovenatum*. The compound demonstrated complete viral inhibition against Herpes simplex virus I (HSV-I) at 100 μg/ml. In its antiviral activity it resembled the more active crisamicin A1.[5] The monomeric compounds **1a** and **1b** showed no activity in the assays.

Structure Elucidation

The structures of the compounds were determined in part by spectral analysis, particularly long- and short-range ^1H-^{13}C heteronuclear correlations (attached proton test and HETCOR), and were confirmed by chemical conversions. An HREI mass spectrum for **1b** provided a molecular ion at *m/z* 358.1060 ($C_{19}H_{18}O_7$, Δ0.3 mmu), implying eleven degrees of unsaturation. An HRFAB mass spectrum of **1c** provided a molecular ion (M+H) at *m/z* 715.2039 ($C_{38}H_{35}O_{14}$, Δ1.2 mmu), which is consistent with a symmetrical dimerization of **1b** (*i.e.*, $2 \times 358 - H_2$). The 360-MHz ^1H NMR spectral data for each of the spectomycins are presented in Table 1, and the 90-MHz ^{13}C NMR data are presented in Table 2. The ^{13}C NMR spectral data are consistent with the mass spectral data, in that nineteen carbon resonances are observed for both **1b** and **1c**, implying (again) dimerization of the former to provide the latter. Compound **1a** was isolated as a minor component from the broth, and provided a molecular ion (HREI-MS) at *m/z* 372.1261 ($C_{20}H_{20}O_7$, Δ2.6 mmu), and twenty signals in the ^{13}C NMR spectrum, including an *O*-CH_3 carbon at 56.1 ppm, implying that it is an *O*-methylated derivative of **1b**.

In the ^1H NMR spectrum of **1b** a series of three *ortho*-coupled aromatic protons was observed at 7.52 (t), 7.15 (d), and 6.83 (d) ppm, and an aromatic

Table 1. ^1H NMR spectral data for the spectomycins.

Proton	Spectomycin		
	1a[a] δ ppm, mult,[c] J (Hz)	**1b**[a] δ ppm, mult,[c] J (Hz)	**1c**[b] δ ppm, mult,[c] J (Hz)
2a	3.05, d, 17.4	3.05, d, 17.6	3.08, d, 17.5
2b	2.82, d, 17.4	2.94, d, 17.6	2.88, d, 17.5
4	5.48, s	5.50, s	5.78, s
5	7.20, d, 8.0	7.15, d, 8.0	7.36, d, 8.3
6	7.50, t, 8.0	7.52, t, 8.0	7.68, d, 8.3
7	6.84, d, 8.0	6.83, d, 8.0	
10	6.88, s	6.89, s	7.12, s
3-CH_3	1.42, s	1.43, s	1.42, s
8-OH		9.75, s	9.82, s
8-OCH_3	4.02, s		
9-OH	16.08, s	16.10, s	15.98, s
2′	5.44, s	5.48, s	5.56, s
3′-OCH_3	3.69, s	3.72, s	3.78, s

[a] In $CDCl_3$. [b] In CD_3COCD_3. [c] Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet.

Table 2. ^{13}C NMR spectral data for the spectomycins.

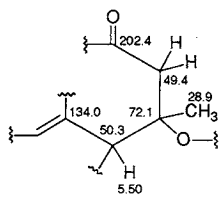
Carbon	m[a]	**1a**[b]	**1b**[b]	**1c**[c]	**2b**
1	s	202.6	202.4	205.2	195.4
2	t	49.7	49.4	50.4	51.7
3	s	71.8	72.1	72.4	71.7
4	d	50.8	50.3	50.9	51.6
4a	s	135.1	134.0	136.9	136.4
5	d	120.0	118.4	118.7	118.4
6	d	131.2	132.7	136.5	130.8
7	d	106.2	111.5	121.0 (s)	111.4
8	s	159.8	158.0	155.7	156.2
8a	s	115.5	112.9	113.5	119.7
9	s	165.7	165.4	166.3	159.6
9a	s	111.2	109.1	110.9	116.7
10	d	117.4	118.0	118.6	123.8
10a	s	139.8	138.9	139.7	138.0
3-CH_3	q	29.1	28.9	29.0	29.3
8-OCH_3	q	56.1			
9-OCH_3	q				64.4
1′	s	172.9	172.9	169.3	169.1
2′	d	94.7	95.1	95.8	95.2
3′	s	174.8	174.4	173.6	172.6
1′-OCH_3	q				51.5
3′-OCH_3	q	56.4	56.4	56.5	56.1

[a] Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet. [b] In $CDCl_3$. [c] In CD_3COCD_3.

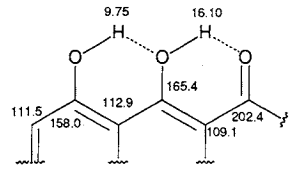
singlet was observed at 6.89 ppm. Two singlets at 5.50 and 5.48 ppm were thought at first to correspond to two olefinic resonances, but a heteronuclear correlation experiment indicated that the proton at 5.50 ppm was actually an aliphatic methine proton with a very odd, downfield chemical shift (*vide infra*). Also observed were resonances for *O*-methyl (3.72 ppm) and *C*-methyl (1.43 ppm) singlets, a pair of AB doublets (3.05 and 2.82 ppm), and two phenolic hydroxyls (9.75 and 16.10 ppm). The chemical shift at 16.10 ppm suggested a strongly chelated hydroxyl proton from the enol form of a 1,3-diketone or a *peri*-hydroxy naphthoquinone. The infrared spectrum (1682 cm^{-1}) and acidic behavior of the compound suggested the presence of a conjugated carboxylic acid, although the proton for this functional group was not observed. In all, the ^1H NMR spectrum could account for a total of sixteen of the eighteen protons, and another two protons (–OH and –COOH) may have been buried in the baseline. The lack of additional coupling information did not permit assignment of further structural units.

The ^{13}C NMR spectrum of **1b** showed resonances for a ketone (202.4 ppm), an acid (172.9 ppm), and the α-carbon of an enol methyl ether (174.4 ppm). An olefinic carbon far upfield at 95.1 ppm was assigned to the β-carbon of the enol ether. In addition, ten resonances for aromatic-type carbons, two of which were phenol-bearing carbons (165.4 and 158.0 ppm) and four of which were substituted by hydrogen (132.7, 118.4, 118.0 and 111.5 ppm), implied a naphthalene ring system with two oxygen and two carbon substituents. An aliphatic methine carbon (50.3 ppm), a methylene carbon (49.4 ppm), an *O*-methyl (56.4 ppm), a *C*-methyl (28.9 ppm), and an oxygen-substituted quaternary carbon (72.1 ppm) accounted for the remaining resonances, for a total of nineteen carbons. The naphthalene ring, ketone and carboxylate carbonyls, and the enol ether accounted for ten of the eleven degrees of unsaturation, suggesting the presence of another ring.

Unequivocal assignments for the resonances could be obtained from short-range and long-range



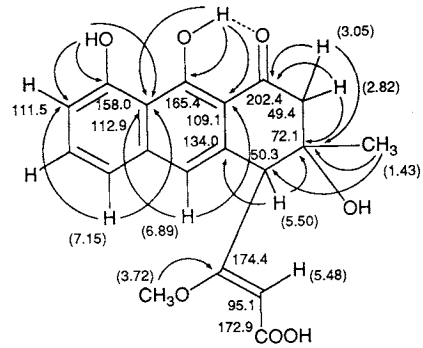
Fragment A



Fragment B

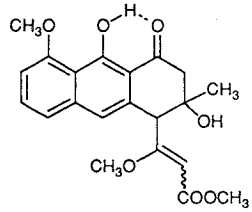
heteronuclear correlations. Assignment of the short-range correlations for the *O*-methyl, *C*-methyl, methylene, methine, and four aromatic resonances was straightforward. The proton singlets at 5.50 and 5.48 ppm were clearly attached to rather distinct carbons (an aliphatic methine carbon at 50.3 ppm and an olefinic carbon at 95.1 ppm, respectively). The long-range HETCOR spectrum provided correlation between the proton *O*-methyl singlet at 3.72 ppm and the carbon resonance at 174.4 ppm (α-carbon of the enol ether). The proton *C*-methyl absorption at 1.43 ppm was correlated with carbon resonances for the methine (50.3 ppm), methylene (49.4 ppm), and quaternary (72.1 ppm) carbons. Both of the methylene protons (3.05 and 2.82 ppm) and the aliphatic methine proton (5.50 ppm) were also correlated with the quaternary carbon. Hence, an aliphatic carbon framework consisting of a quaternary carbon attached to a methyl, a methine, a methylene, and an oxygen substituent could be constructed. Because the methine proton at 5.50 ppm was correlated with the aromatic carbon at 134.0 ppm, and the methylene protons with the ketone carbon at 202.4 ppm, Fragment A can be drawn.

Scheme 1. Long-range proton-carbon heteronuclear correlations for **1b** with relevant chemical shift data.



Further evidence includes correlations between the two phenolic hydrogen at 16.1 ppm and the aromatic resonances at 109.1, 112.9, and 165.4 ppm, and the other phenolic hydrogen, at 9.75 ppm, with the carbon resonances at 111.5, 112.9, and 158.0 ppm. Since the phenolic hydrogen at 16.1 ppm must be hydrogen-bonded to the ketone, Fragment B is implied. All of the relevant long-range correlations are summarized in Scheme 1. From a few remaining correlations and proton coupling systems the entire tetrahydroanthracene ring system can be constructed, with the 3-carbon sidechain assigned as the substituent at C-4 by default. The methoxyl (correlated with the disubstituted olefinic carbon at 174.4 ppm) and the olefinic proton, on the olefinic carbon at 95.1 ppm and not coupled to H-4, must be on opposite ends of the double bond. The carboxyl group must then be attached to the β-position of the enol ether group, as shown, and this is supported by the chemical shift of the olefinic β-proton at 5.48 ppm. The final proposed structure for the monomeric compound is shown as **1b**. The stereochemistry of the ring substituents and that of the olefinic double bond have not yet been rigorously determined.

The structures for **1a** and **1c** follow from a comparison of their spectral features with those of **1b**. Compound **1a** contains one extra *O*-methyl group relative to **1b** as evidenced by EI-MS (molecular ion at *m/z* 372), by the extra *O*-methyl resonance at 4.02 ppm in the ^{1}H NMR spectrum, and by the *O*-methyl



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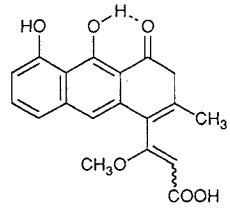

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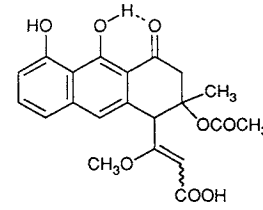

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signal at 56.1 ppm in the ^{13}C NMR spectrum. The methyl ether is located at C-8 because the strongly chelated enolic hydrogen of a 1,3-diketone (H-9) is still observed at 16.1 ppm in the ^{1}H NMR spectrum.

The structure of **1c** is assigned as a symmetrical dimer **1b**. A FAB mass spectrum of **1c** indicated an M+H ion at *m/z* 715 (*i.e.*, M=714=(2×358, **1b**)−2H). All of the resonances for the two compounds are the same, with the exception of one fewer aromatic proton in the proton spectrum of **1c**: the resonance for H-7 in **1b** (at 6.83, furthest upfield of the four) is missing for **1c**, and slight downfield shifts for H-5 and H-6 (both now doublets) are observed. In addition, a downfield shift to 121.0 ppm for the resonance for C-7 is observed in the carbon spectrum of **1c**.

Chemical Conversions

Treatment of **1b** with ethereal diazomethane leads to a mixture of three products, the major one (**2**) being derived from the addition of two methyl groups (EI-MS, *m/z* 386). By proton and carbon NMR, the new methyl groups can be assigned as a methyl ester and a methyl ether. That the methyl ether is located at C-9 is indicated by the lack of a strongly hydrogen-bonded phenol resonance at 16.1 ppm in the ^{1}H NMR spectrum of the compound, as well as by the large change in the chemical shift of the C-1 carbonyl resonance (to 195.4 ppm). The position of this methyl ether is, therefore, quite distinct from that of the methyl ether in the natural product **1a**. The other two products of the reaction were not well characterized.

Treatment of **1b** with methanol and catalytic sulfuric acid resulted in the formation of a single compound (**3**) that proved to be a dehydration product (EI-MS, *m/z* 340). Because the resonance for the H-4 proton disappears and the resonance for the C-3 methyl group shifts downfield to 1.62 ppm, the dehydration has occurred across the C-3, C-4 bond. Tautomerization of the ketone to the enol (providing an anthracene skeleton) does not occur.

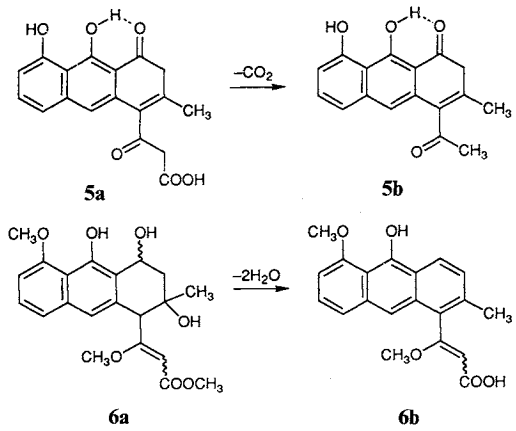
Acetylation of **1b** with acetic anhydride and triethylamine, followed by aqueous extractive workup, provided a monoacetate (**4**). That the acetyl group was introduced on the C-3 hydroxyl is evidenced by the retention of both phenol resonances at 16.1 and 9.8 ppm in the proton NMR spectrum and by a slight downfield shift of the C-3 methyl group to 1.46 ppm. Upon attempted purification of the compound by silica gel chromatography, the acetate was converted to the same compound obtained from the methanolysis reaction (**3**).

Attempted cleavage of the *O*-methyl ether of **1b** with acetic acid and 48% aqueous hydrobromic acid provided an unstable, acidic compound which showed by FAB-MS a highest-mass fragment ion at *m/z* 282 (M−CO_2). Attempted purification by silica gel chromatography led to the quantitative conversion of the original product into a nonpolar compound which showed a molecular ion (FAB-MS) at *m/z* 283 (M+H). This sequence of reactions is consistent with an acid-catalyzed dehydration and cleavage of the methyl enol ether to the β-ketoacid **5a**, which then decarboxylates to provide the methyl ketone **5b**.

Finally, a reduction of **1a** with sodium borohydride in methanol, followed by a mildly acidic workup, provided the anthracene derivate **6b**. The product presumably arises from reduction of the ketone to the dihydroxy compound **6a**, followed by a double dehydration to the anthracene **6b**.

Biosynthetic Studies

S. spectabilis was grown as described above in 5 liters of Medium I in a jar fermentor. Sodium [1,2-$^{13}C_2$]acetate (700 mg, 90% ^{13}C) was dissolved in 20 ml of water, sterilized by membrane filtration, and fed to the culture in two equal portions at 31 and 58 hours after inoculation. At 72 hours, the fermentation was worked up as described above to provide 53 mg of **1b** and 47 mg of **1c** (along with another 100 mg of partially purified **1c**). The ^{13}C NMR spectra of both compounds showed the ^{13}C-^{13}C coupling patterns expected for intact incorporation of acetate units into a nonaketide biogenetic



Scheme 2. Incorporation of [1,2-$^{13}C_2$]acetate into **1b** and **1c**.

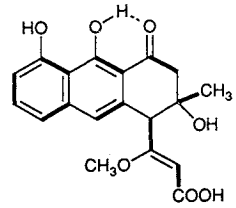


Table 3. Coupling constants for incorporation of [1,2-$^{13}C_2$]acetate and enrichment factors for incorporation of [1-^{13}C]acetate into **1b** and **1c**[a].

Carbon	**1b**[b]			**1c**[c]	
	δ ppm	$^1J_{C-C}$	Enrichment[d]	δ ppm	$^1J_{C-C}$
3-Me	28.9	40.2	1.0	29.0	38.6
3	72.4	39.8	2.9	72.4	40.0
2	51.0	40.5	1.1	51.1	40.6
1	205.2	40.4	2.1	205.1	40.9
9a	110.8	68.8	1.6	111.1	65.5
9	165.8	63.8	3.6	(165.0)[e]	
8a	111.7	56.1	2.0	113.5	62.8
8	158.7	62.2	3.3	155.5	62.1
7	111.6	56.7	1.0	121.0	57.9
6	133.3	56.2	2.2	136.4	57.8
5	119.3	56.5	0.8	118.6	56.0
10a	140.2	56.6	4.9	139.7	55.9
10	118.6	66.3	0.9	118.9	65.8
4a	137.0	65.9	2.8	136.9	66.4
4	50.2	48.7	0.9	50.5	48.6
3′	173.5	48.8	3.3	173.6	48.7
2′	95.9	78.6	0.8	95.9	78.6
1′	169.3	78.5	3.6	169.5	78.6
3′-OMe	56.5		1.0	56.5	

[a] In CD_3COCD_3. [b] 16 K data size with zero filling to 32 K over 16393 Hz spectral width. [c] 64 K data size over 16393 Hz spectral width. [d] Ratio of peak heights, normalized to the 3′-*O*-methyl as 1.0. [e] Observed as a broad, poorly resolved signal.

precursor, as summarized in Table 3. A similar experiment was done with sodium [1-^{13}C]acetate (615 mg in two equal portions at 48 and 60 hours after inoculation), providing 9.8 mg of **1b** and 2.1 mg of **1c**. The enrichment factors observed in the ^{13}C NMR spectrum for **1b** are also summarized in Table 3. The incorporation pattern summarized in Scheme 2 provides additional evidence for the proposed structure, as the carbon skeleton can be traced from well-established carbon signal assignments by pairing signals with identical coupling constants. The only unlabeled carbon signal (the 3'-OMe carbon) should be derived from the *S*-methyl of methionine. The dimeric structure presumably arises from two monomeric species *via* oxidative phenolic coupling.

Experimental

General Methods

^{1}H and ^{13}C NMR spectra were obtained on either a General Electric QE-300 at 300 MHz or a Nicolet NT-360 spectrometer at 360 MHz. Heteronuclear correlation experiments were obtained on a General Electric GN-500 spectrometer at 500 MHz with the software (CSCM and CSCMLR experiments) supplied by the manufacturer. IR spectra were obtained on an IBM IR-30S Fourier transform spectrophotometer, and UV spectra on a Perkin-Elmer Lambda 3 UV spectrophotometer. EI-MS was performed on a Finnigan-MAT CH5 instrument at 70 eV and FAB-MS on either a VG Analytical ZAB-SE or a VG 70-SE 4F four sector instrument with xenon fast atom gun using a dithiothreitol-dithioerythritol matrix ("Magic Bullet").[6] Melting points were obtained on a Reichert hot stage melting point apparatus. Optical rotations were obtained on a JASCO DIP-360 digital recording optical rotation instrument in 100-mm cells. Sodium [1,2-$^{13}C_2$]acetate was purchased from ICN Biomedicals, and sodium [1-^{13}C]acetate from MSD Isotopes. Thin layer chromatography was on silica gel plates with indicator (0.25 mm, Kieselgel 60 F-254, Merck, Darmstadt). Silica gel for chromatography (40~63 μm) was purchased from Merck, and Sephadex LH20 was obtained from Pharmacia.

Culture Conditions

S. spectabilis (UC 2294) was maintained in soil stocks and agar slants (10.0 g of maltose, 5.0 g of DIFCO tryptone, 1.0 g of K_2HPO_4, 1.0 g of NaCl, a trace of $FeSO_4$, and 15.0 g of agar per liter of distilled water). A seed culture in 100 ml of Medium III (20.0 g of Sheffields NZ-Amine B, 10.0 g of glucose, 2.5 g of K_2HPO_4, 1.5 g of K_2HPO_4, and 10.0 ml of La Choy soy sauce per liter of distilled water) in a 500-ml wide-mouthed Erlenmeyer flask was inoculated with a few grains of soil stock and incubated at 32°C and 250 rpm in a New Brunswick G-25 rotary incubator for 48 hours. A 10.0-ml aliquot of the seed medium was then inoculated into 10 liters of synthetic Medium I in a 14-liter glass jar (New Brunswick Microferm), and incubated at 30°C with 250 rpm agitation and an aeration rate of 5 liters/minute for 4 days. The entire culture broth was then inoculated into 200 liters of Medium I in a 250-liter New Brunswick fermentor at 30°C, with 250 rpm agitation rate and 100 liters/minute aeration rate, and incubated for 8 days.

Isolation and Purification

By the end of the fermentation the pH had dropped to 3.9. The whole beer was batch-extracted with ethyl acetate (2 × 3.0 liters per 12-liter batch). The combined organic extracts were concentrated to 500 ml by rotary evaporation at 40°C, washed with water (200 ml), and back-extracted with 5% sodium bicarbonate (4 × 100 ml). The combined bicarbonate layers were reacidified to pH 4.0 with 6 N hydrochloric acid and extracted again with ethyl acetate (3 × 200 ml). The combined organic layers were washed with water (50 ml) and brine (2 × 50 ml), then dried (Na_2SO_4). After removal of the ethyl acetate, the crude, dark brown extract was dissolved in 20 ml of chloroform and flash chromatographed on silica, from which the main yellow band was eluted with chloroform - methanol, 9 : 1. After evaporation of the solvent, the residue was dissolved in 35 ml of chloroform, applied to a column of Sephadex LH-20 (300 ml), and eluted with a chloroform - methanol gradient. Three main bands eluted at 1,000 ml of chloroform (fraction A), 1,500 ml of chloroform (fraction B), and 500 ml of 95 : 5 chloroform - methanol (fraction C).

Fraction A was purified by reprecipitation from ethyl acetate-hexane to provide 9.1 mg of a bright yellow powder (**1a**). Fraction B was purified by silica gel chromatography with a chloroform-methanol step gradient to yield 170 mg of a dark yellow powder (**1b**). Fraction C was purified by reprecipitation from ethyl acetate-hexane to provide 324 mg of a dark, orange-brown powder (**1c**).

Spectomycin A1 (**1a**): mp 118~122°C; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); IR (Nujol) 1684, 1616, 1576, 1456, 1398, 1373, 1267, 1155, 1097, 843 cm^{-1}; UV (MeOH) λ_{max} (log ε) 402 (3.95), 295 (3.60), 284 (3.66), 258 (4.54), 215 (4.53); UV (MeOH-NaOH) λ_{max} (log ε) 422 (4.01), 335 (3.51), 258 (4.46), 215 (4.45); EI-MS *m/z* 372, 354, 322, 310, 294, 270, 254, 165, 152, 144. HREI-MS. Found: *m/z* 372.1235 (M). Calcd for $C_{20}H_{20}O_7$: *m/z* 372.1261.

Spectomycin A2 (**1b**): mp 136~138°C; $[\alpha]_D^{25}$ −225° (*c* 0.25, $CHCl_3$); ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); IR (Nujol) 3450, 1682, 1630, 1447, 1414, 1379, 1333, 1271, 1196, 1165, 1109, 1049, 991, 835, 808, 796, 771, 736, 702 cm^{-1}; UV (MeOH) λ_{max} (log ε) 399 (3.91), 260 (4.54), 215 (4.49); UV (MeOH-NaOH) λ_{max} (log ε) 420 (4.03), 260 (4.43), 215 (4.53); EI-MS *m/z* 358, 340, 326, 308, 296, 280, 256, 240, 165. HREI-MS. Found: *m/z* 358.1057 (M). Calcd for $C_{19}H_{18}O_7$: *m/z* 358.1060.

Spectomycin B1 (**1c**): mp 118~122°C; ^{1}H NMR (Table 1); ^{13}C NMR (Table 2); IR (Nujol) 3400, 1684, 1618, 1506, 1406, 1327, 1242. 1192, 1155, 1107, 1051, 833 cm^{-1}; UV (MeOH) λ_{max} (log ε) 430 (4.14), 269 (4.43), 224 (4.37); FAB-MS *m/z* 715 (M+H). HRFAB-MS. Found: *m/z* 715.2039 (M+H). Calcd for $C_{38}H_{35}O_{14}$: *m/z* 717.2027.

Methylation of **1b**. Excess ethereal diazomethane was added to 21.3 mg of **1b** in 1.0 ml of methanol at 0°C and the mixture was stirred at 25°C for 2 hours. The solvent was evaporated under a stream of nitrogen to yield 23.2 mg of a yellow residue as a mixture of compounds. The major product (10.4 mg) was isolated by silica gel chromatography (99:1 chloroform-methanol), and was shown to be 9-*O*-methylspectomycin A2 methyl ester (**2**), mp 128~130°C; ^{1}H NMR ($CDCl_3$) δ 9.92 (1H, s, C-8 -O*H*), 7.45 (1H, t, *J*=7.8 Hz, H-6), 7.31 (1H, s, H-10), 7.22 (1H, d, *J*=7.9 Hz, H-5), 6.89 (1H, d, *J*=7.7 Hz, H-7), 5.52 (1H, s, H-2'), 4.06 (3H, s, 9-OCH_3), 3.78 (3H, s, 3'-OCH_3), 3.64 (3H, s, 1'-OCH_3), 2.98 (1H, d, *J*=17.4 Hz, H-2a), 2.78 (1H, d, *J*=17.4 Hz, H-2b), 1.41 (3H, s, 3-CH_3); ^{13}C NMR (Table 2); IR (Nujol) 1686, 1653, 1628, 1437, 1346, 1269, 1145, 1105, 850 cm^{-1}; UV (MeOH) λ_{max} (log ε) 380 (3.2), 258 (4.0), 220 (4.1); FAB-MS *m/z* 387 (M+H), 369 ((M+H)−H_2O), 337 ((M+H)−H_2O−CH_3OH). HRFAB-MS. Found: *m/z* 387.1426. Calcd for $C_{21}H_{23}O_7$: *m/z* 387.1408.

Methanolysis of **1b**. A solution of 4.1 mg of **1b** in 3.0 ml of methanol and 0.2 ml of concentrated sulfuric acid was stirred overnight at 25°C. The mixture was cooled to 0°C, and a light yellow solid precipitated from the solution. After filtration and washing with cold methanol, 2.4 mg of a yellow powder, 3,4-didehydro-3-deoxyspectomycin A2 (**3**), was obtained, mp 144~146°C; ^{1}H NMR ($CDCl_3$) δ 15.9 (1H, br s, 9-OH), 9.67 (1H, s, 8-OH), 7.54 (1H, t, *J*=7.8 Hz, H-6), 7.19 (1H, d, *J*=7.8 Hz, H-5), 7.17 (1H, s, H-10), 6.93 (1H, d, *J*=7.8 Hz, H-7), 5.28 (1H, s, H-2'), 3.86 (3H, s, 3'-OCH_3), 3.33 (1H, d, *J*=17.2 Hz, H-2a), 2.93 (1H, d, *J*=17.2 Hz, H-2b), 1.62 (3H, s, 3-CH_3); IR (Nujol) 1697, 1628, 1412, 1366, 1345, 1260, 1229, 1188, 1163, 1107, 1064, 1010, 856, 822, 629 cm^{-1}; UV (MeOH) λ_{max} (log ε) 407 (3.77), 267 (4.35), 220 (4.38); EI-MS *m/z* (% rel int) 340 (13.3), 322 (12.6), 308 (100.0), 148 (53.4); FAB-MS *m/z* 341 (M+H), 323 ((M+H)−H_2O), 297 ((M+H)−CO_2), 241 ((M+H)−$C_4H_4O_3$). HRFAB-MS. Found: *m/z* 341.1031. Calcd for $C_{19}H_{17}O_6$: *m/z* 341.1037.

Acetylation of **1b**. Triethylamine (0.2 ml) was added to 6.1 mg of **1b** in 1.5 ml of acetic anhydride and the mixture was stirred overnight at 25°C. Excess reagent was quenched by the addition of methanol at 0°C, and the mixture was poured into 20 ml of water. The aqueous suspension was extracted with 10 ml of ethyl acetate, and the organic layer was washed successively with 5 ml each of 5% aqueous hydrochloric acid, water, and brine. After drying (Na_2SO_4) and evaporation, the dark orange product was dissolved in 1 ml of ethyl acetate and 3 ml of hexane was added. A small amount of dark red precipitate was filtered off and discarded, and the filtrate was evaporated to give 4.8 mg of a light yellow powder (**4**), mp 133~135°C; ^{1}H NMR ($CDCl_3$) δ 16.08 (1H, s, 9-OH), 9.75 (1H, s, 8-OH), 7.31 (1H, t, *J*=7.8 Hz, H-6), 7.16 (1H, d, *J*=7.9 Hz, H-5), 6.95 (1H, s, H-10), 6.88 (1H, d, *J*=7.8 Hz, H-7), 5.45 (1H, s, H-2'), 5.39 (1H, s, H-4), 3.75 (3H, s, 3'-OCH_3), 3.08 (1H, d, *J*=17.7 Hz, H-2a), 2.82 (1H, d, *J*=17.7 Hz, H-2b), 2.29 (3H, s, CH_3COO–), 1.46 (3H, s, 3-CH_3); FAB-MS *m/z* 401 (M+H). The compound was not further characterized due to its low purity at this point. Attempted purification of the compound by silica gel chromatography led to the elimination of acetic acid to provide a product identical with that obtained from the methanolysis

(3).

Acid hydrolysis of **1b**. A 6.2-mg portion of **1b** was dissolved in a mixture of 2 ml of acetic acid and 1 ml of 48% aqueous hydrobromic acid and the solution was stirred at 25°C overnight. The mixture was then poured into 20 ml of water and extracted with 10 ml of ethyl acetate. The organic layer was washed with 5 ml each of water and brine, then dried (Na_2SO_4). Evaporation of the solvent provided 5.6 mg of a yellow-orange powder which proved to be highly unstable. The product had Rf 0.18 on silica (chloroform - methanol, 9 : 1), and FAB-MS provided a highest-mass fragment ion at *m/z* 282 ($M-CO_2$). Upon standing in solution or chromatography on silica, the compound converted rapidly and quantitatively to a less polar product (Rf 0.9, chloroform - methanol, 9 : 1), whose FAB mass spectrum indicated a molecular ion ($M+H$) at *m/z* 283. The above conversion is consistent with a decarboxylation of the β-keto acid **5a** to the methyl ketone **5b**, mp 132~138°C; ^1H NMR ($CDCl_3$) δ 7.49 (1H, t, J=6.8 Hz, H-6), 6.91 (1H, d, J=6.8 Hz, H-5), 6.88 (1H, d, J=6.8 Hz, H-7), 6.80 (1H, s, H-10), 4.21 (2H, s, H-2), 2.54 (3H, s, 3-CH_3), 2.33 (3H, s, CH_3CO–); IR (Nujol) 1695, 1633, 1610, 1570, 1458, 1360, 1280, 1226, 1180, 914, 854, 769, 748 cm^{-1}; EI-MS *m/z* (% rel int) 282 (62.8), 267 (55.3), 240 (41.7). The compound was not characterized further.

Hydride reduction of **1a**. A solution of 55 mg of sodium borohydride in 3 ml of methanol was added to a solution of 19.2 mg of **1a** in 1.5 ml of methanol at 0°C. After 1 hour at 0°C, another 10 mg of solid sodium borohydride was added, and the mixture was stirred for 1 hour at 25°C. The mixture was acidified with 5 ml of 5% aq hydrochloric acid then poured into 20 ml of water. The aqueous solution was extracted with 10 ml of ethyl acetate, and the organic layer was washed with 5 ml each of water and brine. After drying and evaporation, 16.1 mg of a yellow powder, 3-(9-hydroxy-8-methoxy-3-methylanthracen-4-yl)-3-methoxypropanoic acid (**6b**), was obtained, mp 136~138°C; ^1H NMR ($CDCl_3$) δ 10.13 (1H, s, H-9), 8.34 (1H, d, J=8.9Hz, H-1), 7.52 (1H, s, H-10), 7.38 (1H, d, J=8.5 Hz, H-5), 7.18 (1H, d, J=8.9 Hz, H-2), 7.14 (1H, dd, J=8.5, 7.5 Hz, H-6), 6.51 (1 H, d, J=7.5 Hz, H-7), 5.61 (1H, s, H-2'), 4.01 (3H, s, 9-OCH_3), 3.85 (3H, s, 3'-OCH_3), 2.32 (3H, s, 3-CH_3); ^{13}C NMR ($CDCl_3$) δ 171.5 (s), 170.4 (s), 156.4 (s), 151.1 (s), 134.2 (s, 2 C), 130.6 (s), 129.7 (s), 126.4 (d), 124.6 (d), 123.7 (s), 122.3 (d), 118.7 (s), 112.1 (d), 109.8 (s), 101.0 (d), 95.6 (d), 56.4 (q), 55.9 (q), 19.7 (q); IR (Nujol) 1708, 1667, 1624, 1593, 1469, 1269, 1078, 833, 787, 711 cm^{-1}; UV (MeOH) λ_{max} (log ε) 320 (3.0), 256 (3.9), 238 (3.9), 202 (4.0); EI-MS *m/z* (% rel int) 338 (3.09), 307 (7.8), 293 (8.3), 279 (7.8), 265 (6.8); FAB-MS *m/z* 339 ($M+H$), 239 (($M+H$)$-C_4H_4O_3$). HRFAB-MS. Found: *m/z* 339.1224 Calcd for $C_{20}H_{19}O_5$: *m/z* 339.1216.

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References

1) STALEY, A. L. & K. L. RINEHART: Biosynthesis of the streptovaricins: 3-Amino-5-hydroxybenzoic acid as a precursor to the *meta*-C_7N unit. J. Antibiotics 44: 218~224, 1991
2) STALEY, A. L.: Isolation, structure, synthesis and biosynthesis of selected bioactive microbial metabolites. Ph.D. Thesis, University of Illinois, Urbana, IL, 1990
3) TSUJI, N. & K. NAGASHIMA: Studies on julimycins—II. The stereochemistry of julimycin B-II. Tetrahedron 24: 4233~4247, 1968
4) TSUJI, N. & K. NAGASHIMA: Studies on julimycins—VIII. The structures of julichromes Q_{1-7}, Q_{8-8}, Q_{3-8}, Q_{3-3} and Q_{1-9}. Tetrahedron 27: 1557~1563, 1971
5) LING, D.; L. S. SHIELD & K. L. RINEHART, Jr.: Isolation and structure determination of crisamicin A, a new antibiotic from *Micromonospora purpureochromogenes* subsp. *halotolerans*. J. Antibiotics 39: 345~353, 1986
6) WITTEN, J. L.; M. H. SCHAFFER, M. O'SHEA, J. C. COOK, M. E. HEMLING & K. L. RINEHART: Structures of two cockroach neuropeptides assigned by fast atom bombardment mass spectrometry. Biochem. Biophys. Res. Commun. 124: 350~358, 1984