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Seongomycin: A New Sulfur-containing Benzo[*b*]fluorene Derived from Genes Clustered with those for Kinamycin Biosynthesis

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Abstract: *Streptomyces lividans* ZX7 transformed with a ~40 KB fragment of *S. murayamaensis* DNA produces the kinamycin biosynthetic intermediates kinobscurinone (**1**) and stealthin C (**2a**), as well as seongomycin (**3**), which had been previously observed in *S. murayamaensis* UV mutant MC1.
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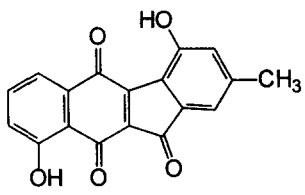
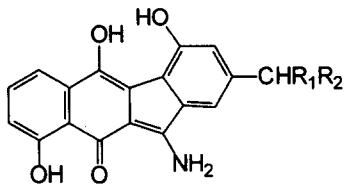
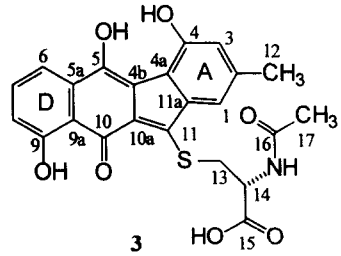
As part of our effort to eventually study the cyclases responsible for generating the angular ring system as well as enzymes that modify the angucycline class of antibiotics,[1] we have recently cloned three angucyclinone polyketide gene clusters from three *Streptomyces* species.[2,3] The genes responsible for the biosynthesis of the kinamycins, a highly modified angucyclinone,[4,5] were cloned from *S. murayamaensis* by screening an *S. murayamaensis* cosmid library with a DNA fragment from the β-ketoacyl synthase gene of the actinorhodin pathway (*actI*).[6] Fourteen cosmid clones having portions of the desired gene cluster were transferred into *S. lividans* ZX7[7] by transformation.[8] Photodiode-array-detected HPLC[9] revealed that four of these fourteen transformants produced varying amounts of kinobscurinone (**1**)[10] and stealthin C (**2a**),[11] which have recently been shown to be intermediates in kinamycin biosynthesis, as well as another metabolite, seongomycin (**3**), previously observed as a minor component in extracts of *S. murayamaensis* UV mutant MC1.[9] This was a major metabolite in the transformants, although it had not been observed from the wild-type organism under various culture conditions. No kinamycins were observed.



1



2 a R_1 = H, R_2 = H
2 b R_1 = H, R_2 = OH
2 c R_1 = R_2 = O



3

For a typical isolation, a 500 mL GPS production culture[3] was harvested at 48 h by centrifugation (10,000 g, 15 min), and the supernatant was extracted with EtOAc (400 mL). The layers were separated and

the lower layer was acidified to pH 3.4 with 1 N HCl and extracted with EtOAc (500 mL). The upper layer was filtered through Celite, dried over Na_2SO_4 and concentrated to give 149 mg of extract. Purification by Sephadex LH-20 chromatography (2.5 X 49 cm, Me_2CO/MeOH, 1:1) gave 12 mg of **3** as a dark purple solid.[12]

A molecular formula of $C_{23}H_{19}NO_7S$ for **3** was determined by HRFABMS,[13] indicating 15 double bond equivalents. Initially only 21 signals were observed in the ^{13}C NMR spectrum of **3**, and several of the ^1H NMR signals were broad and lacking definition. The addition of a drop of TFA-*d* to the solution significantly improved the spectral dispersion, allowing all 23 of the carbon resonances to be observed and the coupling patterns for two of the broad ^1H NMR signals to be discerned. An HMQC NMR experiment established the connectivity of 14 protons to 9 carbons and suggested 2 methyl, 1 methylene, 6 methine, and 14 quaternary carbons. The five remaining protons were exchangeable, and only two, a doublet at δ 8.23 and a singlet at 13.6, attributable to amide and hydrogen-bonded protons, respectively, were observed in the ^1H NMR spectrum.

HMBC experiments and ^1H-^1H couplings were used to determine C-C connectivities. A three-proton spin system, δ 7.30 (1H, d, 8 Hz, H-6), 7.50 (1H, t, 8 Hz, H-7), and 6.91 (1H, d, 8 Hz, H-8), suggested a 1,2,3-trisubstituted aromatic ring. HMBC correlations of the hydrogen-bonded proton (9-OH) to C-8 and quaternary carbon signals at 163.1 (C-9) and 116.0 (C-9a) permitted the placement of a phenol function at C-9. Cross peaks from H-7 to C-9 and a carbon resonating at δ 133.9 (5a), H-8 to C-6, C-9, and C-9a, and H-6 to C-7, C-8, and C-9a fully elaborated the ring D. A 3-bond coupling was also observed from H-6 to a quaternary heteroatom-substituted sp^2 carbon resonating at δ 148.2 (C-5), allowing the linking of ring D to ring C.

For ring A, broad signals at δ 7.12 (H-1) and 6.70 (H-3) both showed HMBC correlations to a signal at δ 120.5 (4a), the carbon to which the other was attached (C-3 and C-1), and a methyl group resonating at δ 21.3 (C-12). The H-12 hydrogens showed C-H couplings to C-1 and C-3 and a carbon signal at δ 138.7, which was assigned as C-2. A phenol function was placed at C-4 due to a 2-bond correlation from H-3 to a carbon signal at δ 149.2 and to the chemical shifts of H-1 and H-3. A strong correlation from H-1 to a carbon signal at δ 146.1 (C-11) suggested a 3-bond coupling, and therefore a link for ring A to ring B could be established. No C-H couplings to C-11a were observed.

The ^1H and ^{13}C NMR chemical shifts of C-13 to C-17 suggested that **3** had an *N*-acetyl cysteine sub-unit. The assignments were fully supported by HMBC data (Table 1) and a fragment ion in the FABMS of **3** which corresponded to the loss of $C_5H_8NO_3$.[14] HMBC correlations of the H-13 hydrogens to C-11 unambiguously established the position at which it connected to the chromophore.

Four carbon atoms to which no strong heteronuclear couplings were observed remained to be placed. A weak 4-bond correlation from H-3 to a carbon signal at δ 114.1 was observed. The carbon atom was placed at C-4b instead of C-11a because of its chemical shift. Ring D with a carbonyl group at C-10 is a common structural motif in angucyclines,[1] so the carbon resonating at δ 184.0 was assigned to C-10 to account for the H-bonding of 9-OH. Finally, the two remaining carbons were assigned to C-10a and C-11a to give a benzo[*b*]fluorene chromophore analogous to the stealthins (**2a-c**),[11,15] thus completing the structure of **3**.

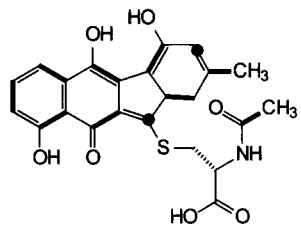
A biosynthetic incorporation of [1,2-$^{13}C_2$]acetate served to confirm the assignments, the structure, and that the new metabolite was indeed polyketide-derived. A mixture of sodium [1,2-$^{13}C_2$]acetate (260 mg) and sodium acetate (260 mg) was pulse-fed at 18, 24, 36, and 48 h to a total of 1 L of production culture under typical culture conditions. Work-up and isolation gave 30 mg of **3a**. A 2D INADEQUATE NMR experiment and $^1J_{c\text{-}c}$ values of the sample clearly indicated which carbons were incorporated from intact acetate units (Table 1). The labeling pattern of the chromophore is the same as that observed for other

benzo[*b*]fluorenes.[16,17] The expected coupling for carbons of the cysteine side chain was not observed, presumably due to the presence of a sizable serine/cysteine pool at the time of the addition of the labeled acetate.

Table 1. NMR Data for **3** (DMSO-d_6 + 1 drop TFA-*d*)

no.	^{13}C	^{1}H	HMBC	INADEQUATE ($^{1}J_{C,C}$, Hz)
1	117.4	7.12 (1H, bs)	H-3, H_3-12	C-11a (63)
2	138.7		H_3-12	C-12 (44)
3	117.1	6.70 (1H, bs)	H-1, H_3-12	
4	149.2		H-3	C-4a (73)
4a	120.5		H-1, H-3	C-4 (73)
4b	114.1		H-3 (weak 4-bond)	C-5 (76)
5	148.2		H-6	C-4b (76)
5a	133.9		H-7	C-6 (59)
6	116.1	7.30 (1H, d, 8 Hz)	H-7, H-8	C-5a (59)
7	135.9	7.50 (1H, t, 8 Hz)	H-6	C-8 (57)
8	119.7	6.91 (1H, d, 8 Hz)	H-6, 9-OH*	C-7 (57)
9	163.1		H-7, H-8, 9-OH*	C-9a (63)
9a	116.0		H-6, H-8, 9-OH*	C-9 (63)
9-OH		13.6 (1H, bs)*		
10	184.0			C-10a (63)
10a	128.4			C-10 (63)
11	146.1		H-1, H_2-13	
11a	141.8			C-1 (63)
12	21.3	2.27 (3H, s)	H-1, H-3	C-2 (44)
13	34.8	3.86 (1H, dd, 13, 4 Hz) 3.53 (1H, dd, 13, 8 Hz)	H-14	
14	52.6	4.43 (1H, td, 8, 4 Hz)	H_2-13	
15	171.9		H_2-13, H-14	
16	169.7		H-14, H_3-17, NH*	C-17 (51)
17	22.3	1.73 (3H, s)		C-16 (51)
NH		8.23 (1H, d, 8 Hz)*		

* Without TFA-*d*.



3 a

To determine the absolute stereochemistry at C-14 of **3**, 1 mg was reduced with Raney Ni in MeOH overnight. The mixture was filtered and concentrated to give crude *N*-acetyl Ala, which was hydrolyzed with 1 N HCl for 3 h to give Ala. The residue obtained by concentration was treated with Marfey's reagent [*N*α-(2,4-dinitro-5-fluorophenyl)-L-alanamide].[18] Comparison by HPLC [ODS, MeCN/TEAP (0.05 M, pH 3), 33:67, 2 mL/min] of the derivative to Marfey's derivatives of L-Ala (t_R = 4.6 min) and D-Ala (t_R = 7.5 min) indicated the Ala was primarily of L configuration, and therefore, that stereochemistry at C-14 is *R*. Some D-Ala was also detected, presumably being formed during the reduction/hydrolysis of **3**.

The *N*-acetyl cysteine moiety has been rarely found in *Streptomyces*-produced polyketides, but is also found in the angucyclinones cysfluoretin[19] and WS009 A.[20] Recently mycothiol, an *N*-acetyl cysteine

glycoside found in all *Streptomyces* species for which it has been assayed, has been proposed to serve a function analogous to glutathione in cellular oxygen regulation.[21] Although the relationship of **3** to the biosynthesis of the kinamycins is as yet unknown, it is clear that the genes unique for establishing the *N*-acetyl cysteine side chain resides with those for the kinamycin pathway.

ACKNOWLEDGEMENTS

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12. Seongomycin (**3**): IR (NaCl, solid) ν_{max} 3330 (br), 1670, 1597, 1460, 1318, 1230 cm^{-1}; UV (MeOH) λ_{max} (log ε) 284 (4.4), 302sh (4.3), 350 (3.9), 476 (3.5), 504sh (3.4) nm; CD (MeOH) Δε (nm) 2.5 (250), 1.9 (264sh), -1.0 (290), 0.88 (327), 0.66 (362), -0.66 (412). Solutions of **3** where too highly colored to obtain an optical rotation.
13. HRFABMS (positive ion): *m/z* calculated for $C_{23}H_{20}NO_7S$ $(M+H)^+$, 454.0960; observed, 454.0978.
14. HRFABMS (positive ion): *m/z* calculated for $C_{18}H_{12}O_4S$ $(M-C_5H_8NO_3)^+$, 324.04560; observed, 324.04560.
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