Coumabiocins A−F, Aminocoumarins from an Organic Extract of *Streptomyces* sp. L-4-4

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Received December 25, 2009

Bioassay-directed fractionation of the butanol extract of *Streptomyces* sp. L-4-4, using the hyphae formation inhibition assay of a prokaryotic whole cell, led to the isolation of six new aminocoumarins, coumabiocins A−F (**1**−**6**), along with two known compounds, novobiocin (**7**) and isonovobiocin (**8**). Coumabiocins A−E (**1**−**5**) contain three structural elements, a central 3-amino-7-hydroxycoumarin that is linked at the 3-amino group to a prenylated 4-hydroxybenzoic acid moiety and at the 7-position to an L-noviosyl sugar, while coumabiocin F (**6**) lacks the sugar moiety. Their structures were elucidated by spectroscopic methods including 1D- and 2D-NMR techniques and mass spectrometric analyses. Coumabiocins A−E (**1**−**5**) exhibited significant inhibitory activity against *Streptomyces* 85E and gave a 10−15 mm clear zone of inhibition at 20 μg/disk and a 10 mm bald and a 10 mm clear zone of inhibition at 5 and 10 μg/disk, respectively, whereas coumabiocin F (**6**) was inactive.

Actinomycetes from natural sources are widely recognized to produce secondary metabolites, including many antimicrobials such as erythromycin, streptomycin, and tetracycline, with unusual structures and potent biological activities.[1] Therefore, actinomycetes are considered to be a promising resource for new lead compounds in drug development. During the course of our investigation on the bioactive components of microorganisms belonging to the actinomycete family, we have focused on *Streptomyces* species. We recently isolated new imides from *Streptomyces* sp. H7667, which exhibited significant inhibitory activity against yeast glycogen synthase kinase-3β (GSK-3β).[2] In an attempt to identify inhibitors of protein kinases, we used the hyphae-formation inhibition (HFI) assay of a prokaryotic whole cell to search for protein kinase inhibitors.[3,4] Aerial hyphae formation in *Streptomyces* species requires protein kinase activity, and it has been shown that a variety of kinase inhibitors block this process.[3,5] An organic extract of *Streptomyces* sp. L-4-4 showed inhibitory activity in the HFI assay, giving a 32 mm bald and clear phenotype at a concentration of 80 μg/disk. This preliminary result encouraged us to study *Streptomyces* sp. L-4-4 for protein kinase inhibitors. Analysis of the secondary metabolites produced by the fermentation culture of *Streptomyces* sp. L-4-4 led to the isolation of new aminocoumarins (**1**−**6**).

Results and Discussion

The butanol-soluble extract of fermented *Streptomyces* sp. L-4-4 exhibited inhibitory activity in the HFI assay. This extract was separated by column chromatography eluting with a gradient of $CHCl_3$−MeOH, affording a series of fractions, which were further purified by semipreparative reversed-phase HPLC and/or preparative TLC, yielding eight compounds (**1**−**8**). The structures of the new compounds **1**−**6** were established on the basis of 1D- and 2D-NMR and mass spectrometric analyses, whereas the known compounds, novobiocin (**7**)[6] and isonovobiocin (**8**),[6] were assessed by comparison of their spectroscopic data with those reported in the literature and authentic samples. Comparing the data of **1**−**6** with those of novobiocin, the L configuration of the noviosyl residue can be deduced from the negative value of the specific rotation measured for all the compounds containing this sugar.[6]

Compound **1** was isolated as a white, amorphous solid with the molecular formula $C_{31}H_{36}N_2O_{12}$, as determined from HRESIMS. The UV spectrum exhibited absorption maxima at λ_{max} 210 and 324 nm, characteristic of a coumarin chromophore.[7] The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **1** displayed signals characteristic of a noviosyl sugar at δ_H/δ_C 5.53 (1H, d, J = 3.0 Hz, H-1″)/99.9 (C-1″), 5.33 (1H, dd, J = 9.6, 2.5 Hz, H-3″)/73.1(C-3″), 4.22 (1H, dd, J = 3.0, 2.5 Hz, H-2″)/70.9 (C-2″), 3.58 (1H, d, J = 9.6 Hz, H-4″)/82.8 (C-4″), 1.33 (3H, s, H_3-7″)/28.9 (C-7″), and 1.16 (3H, s, H_3-6″)/23.2 (C-6″) and one methyl ether at δ_H/δ_C 3.55 (3H, s, H_3-8″)/61.8 (C-8″). The low-field chemical shift of H-3″ resulted from the deshielding effect of a carbamoyl moiety. The positions of the OMe and carbamoyl groups were indicated by correlations of H-4″ with C-8″ and of H-3″ with C-9″, respectively, in the HMBC spectrum (Figure 1). The carbon chemical shifts were conclusively assigned on the basis of ^{13}C NMR, DEPT, HMQC, and HMBC experiments. The ^{1}H NMR data (Table 1) showed two sets of aromatic signals: one at δ 7.85 (1H, br s, H-2), 7.81 (1H, d, J = 8.5 Hz, H-6), and 6.78 (1H, d, J = 8.5 Hz, H-5) due to a 1,3,4-trisubstituted benzene ring and the other at δ 7.81 (1H, d, J = 8.5 Hz, H-5′) and 7.10 (1H, d, J = 8.5 Hz, H-6′) due to a 1,2,3,4-tetrasubstituted benzene ring. A singlet signal at δ 2.30 (3H, s) was assigned to a methyl on an aromatic ring (H_3-11′). Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of novobiocin (**7**)[6] suggested the presence of a 3-amino-4,7-dihydroxycoumarin skeleton in the molecule.[6] Four proton signals at δ 4.68 (1H, dd, J = 9.4, 8.7 Hz, H-8), 3.23 (2H, br d, J = 9.4 Hz, H_2-7), 1.28 (3H, s, H_3-11), and 1.23 (3H, s, H_3-10) indicated that **1** also incorporated a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuran in its structure. The connectivities of H-5/H-6, H_2-7/H-8, H-5′/H-6′, H-1″/H-2″, H-2″/H-3″, and H-3″/H-4″ in the COSY spectrum led to the assignment of all protons. The correlations of H_3-11′ to C-7′, C-8′, and C-9′ and of H-5′ to C-4′, C-7′, C-9′, and C-10′ in the HMBC experiments confirmed a 4,7-dihydroxycoumarin framework. The attachment of the noviosyl unit to the coumarin skeleton was established through the HMBC correlation of H-1″ with C-7′, while a prenylated 4-hydroxybenzoic acid moiety was located at the 3′-amino group.[6] The observations of clear HMBC correlations from H_2-7 and H-8 to C-4, as well as from H_2-7, H_3-10, and H_3-11 to C-9, revealed the existence of a ring-closure between O-4 and C-8 and positioning of a hydroxy group at C-9, confirming the presence of a 2-(1-

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10.1021/np900843b
Published on Web 04/12/2010

Table 1. ^{1}H NMR Spectroscopic Data (500 MHz) of Compounds **1**−**6** in CD_3OD (δ in ppm, multiplicities, J in Hz)

no.	1	2	3	4	5	6
2	7.85, br s	7.84, br s	7.89, br s	7.79, d (2.1)	7.80, d (1.8)	7.80, br s
5	6.78, d (8.5)	6.81, d (8.6)	6.82, d (7.6)	6.79, d (8.5)	6.88, d (8.1)	6.84, d (8.6)
6	7.81, d (8.5)	7.81, d (8.6)	7.74, br d (7.6)	7.76, dd (8.5, 2.1)	7.75, dd (8.1, 1.8)	7.75, br d (8.6)
7	3.23, br d (9.4)	3.30, m, 3.04, dd (15.6, 7.6)	2.88, t (6.0)	3.08, dd (16.6, 5.6), 2.79, dd (16.6, 7.2)	2.98, dd (14.2, 6.2), 2.82, dd (14.2, 7.8)	2.98, dd (14.1, 5.1), 2.82, dd (14.1, 7.8)
8	4.68, dd (9.4, 8.7)	4.64, br q (7.6)	1.87, t (6.0)	3.79, dd (7.2, 5.6)	4.39, dd (7.8, 6.2)	4.40, dd (7.8, 5.1)
9		1.95, m				
10	1.23, s	0.99, d (7.6)	1.35, s	1.28, s	4.89, br s, 4.78, br s	4.88, br s, 4.78, br s
11	1.28, s	1.03, d (7.6)	1.35, s	1.34, s	1.81, s	1.81, s
5′	7.81, d (8.5)	7.81, d (8.6)	7.81, d (8.7)	7.81, d (8.7)	7.81, d (8.9)	7.66, d (8.6)
6′	7.10, d (8.5)	7.24, d (8.6)	7.24, d (8.7)	7.10, d (8.7)	7.24, d (8.9)	6.88, d (8.6)
11′	2.30, s	2.31, s	2.31, s	2.30, s	2.31, s	2.26, s
1″	5.53, d (3.0)	5.58, d (1.7)	5.58, br s	5.53, d (2.6)	5.58, d (2.5)	
2″	4.22, dd (3.0, 2.5)	4.24, dd (2.6, 1.7)	4.24, br s	4.22, dd (3.0, 2.6)	4.24, dd (3.9, 2.5)	
3″	5.33, dd (9.6, 2.5)	5.32, dd (10.0, 2.6)	5.32, dd (9.1, 3.1)	5.33, dd (9.7, 3.0)	5.32, dd (10.0, 3.9)	
4″	3.58, d (9.6)	3.58, d (10.0)	3.58, d (9.1)	3.56, d (9.7)	3.58, d (10.0)	
6″	1.16, s	1.14, s	1.14, s	1.16, s	1.14, s	
7″	1.33, s	1.34, s	1.34, s	1.35, s	1.34, s	
8″	3.55, s	3.55, s	3.55, s	3.55, s	3.55, s	

Table 2. ^{13}C NMR Spectroscopic Data (125 MHz) of Compounds **1**−**6**[a] in CD_3OD

no.	1	2	3	4	5	6
1	128.3	129.6	124.8	128.1	127.3	127.3
2	128.1	130.5	131.2	131.2	132.8	132.7
3	125.9	125.6	122.6	120.9	124.2	123.0
4	164.0	163.3	165.4	166.7	161.5	161.6
5	109.3	109.8	118.4	117.6	116.1	116.1
6	129.7	126.2	128.5	128.5	129.2	129.1
7	31.0	34.6	23.3	32.1	38.1	38.1
8	91.2	91.0	33.5	71.0	76.3	76.3
9	72.3	33.2	76.6	78.8	148.6	148.7
10	25.1	18.1	27.1	21.2	111.3	111.3
11	25.3	18.2	27.1	25.9	18.1	18.1
12	169.7	165.3	169.7	169.6	169.8	169.2
2′	158.0	159.0	158.6	157.3	159.0	158.1
3′	99.7	100.0[b]	100.1	99.6	103.4	100.2
4′	174.3	171.5	173.2	174.2	171.8	162.3
5′	123.9	123.2	123.2	123.9	123.2	129.2
6′	110.2	111.7	111.8	110.3	111.7	113.2
7′	157.9	158.3	159.2	158.1	159.1	161.1
8′	114.3	115.0	115.0	114.4	112.1	112.2
9′	153.4	151.8	151.8	153.4	151.8	152.8
10′	118.1	116.8	112.1	118.2	114.9	110.3
11′	8.7	8.6	8.6	8.8	8.6	8.2
1″	99.9	100.0[b]	100.0	99.9	100.0	
2″	70.9	70.9	70.9	70.2	70.9	
3″	73.1	73.0	73.0	73.1	73.0	
4″	82.8	82.6	82.6	82.8	82.6	
5″	79.7	80.0	80.0	79.8	80.0	
6″	23.2	23.2	23.2	23.2	23.2	
7″	28.9	29.0	29.0	29.0	29.0	
8″	61.8	61.9	61.9	61.9	61.9	
9″	159.3	159.2	159.6	159.2	159.2	

[a] Assignments were made using HMQC and HMBC data. [b] Overlapped signals.

hydroxy-1-methylethyl)-2,3-dihydrofuran moiety in the molecule. The relative configuration of the noviosyl moiety was elucidated on the basis of coupling constants and results of NOESY experiments. The vicinal coupling constants $J_{1'',2''} = 3.0$ Hz and $J_{2'',3''} = 2.5$ Hz suggested an equatorial orientation for H-1″ and H-2″, whereas the axial orientation of H-3″ and H-4″ was indicated by a $J_{3'',4''} = 9.6$ Hz. NOESY cross-peaks observed from H-1″ to H-2″ and from H-3″ to H-2″ and H_3-6″ confirmed that the sugar was a noviopyranoside and linked to the aglycone by an α-glycosidic linkage. Thus, compound **1** was assigned the structure indicated and named coumabiocin A.

Compound **2** was isolated as a white, amorphous solid whose molecular formula was a deduced as $C_{31}H_{36}N_2O_{11}$ from an exact mass measurement. The molecular formula indicated that **2** had one less oxygen atom than **1**. The ^{13}C NMR (Table 2) and DEPT spectra showed a total of 31 carbons, attributable to six methyls, one methylene, 11 methines, and 13 quaternary carbons, compatible with a novobiocin-like structure.[6] The full ^{1}H and ^{13}C NMR spectroscopic assignments for **2** based on 2D-NMR experiments are shown in Tables 1 and 2 and indicated that **2** had the same skeleton as **1**, except for the absence of an oxygenated quaternary carbon signal at δ_C 72.3 (C-9) in **1**. The ^{1}H NMR spectroscopic data (Table 1) displayed additional signals at δ 1.95 (1H, m, H-9), 1.03 (3H, d, $J = 7.6$ Hz, H_3-11), and 0.99 (3H, d, $J = 7.6$ Hz, H_3-10), indicating the presence of a $-CH(CH_3)_2$ moiety. The connectivity of H_2-7/H-8, H-8/H-9, H-9/H_3-10, and H-9/H_3-11 in the COSY spectrum confirmed that this $-CH(CH_3)_2$ group was connected to C-8. This finding was supported by the HMBC spectrum of **2**, in which the methine proton H-8 correlated with C-3, C-4, C-10, and C-11. Therefore, the structure of **2** was determined as indicated and named coumabiocin B.

Compound **3** was determined to be an isomer of **2** by HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **3** were partially comparable with those of **1** and **2**, suggesting an aminocoumarin system[6] with a noviosyl moiety linked via a glycosidic bond at C-7′ based on the HMBC correlation of H-1″ with C-7′. The ^{1}H and ^{13}C NMR spectroscopic data differed from those of **1** and **2** by the presence of signals at δ_H/δ_C 2.88 (2H, t, $J = 6.0$ Hz, H_2-7)/23.3, 1.87 (2H, t, $J = 6.0$ Hz, H_2-8)/33.5, and 1.35 (6H, s, H_3-10/H_3-11)/27.1. These data were consistent with a 2,2-dimethyldihydropyran moiety whose location was placed at C-3 and C-4 by HMBC correlations from H_2-7 to C-2, C-3, C-4, C-8, and C-9 and from H_2-8 to C-3, C-7, C-9, C-10, and C-11. Therefore, **3** was determined to have the structure shown as coumabiocin C.

Compound **4** was obtained as a white, amorphous solid and showed the molecular formula $C_{31}H_{36}N_2O_{12}$, as determined by

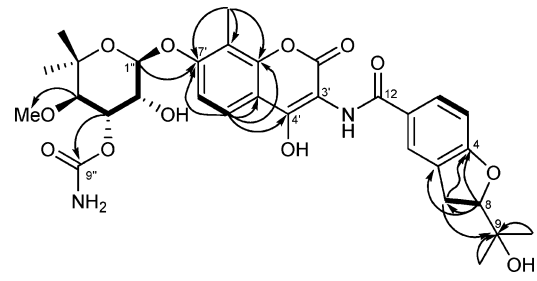


Figure 1. Important ^{1}H−^{1}H COSY (—) and ^{1}H−^{13}C HMBC (→) correlations of compound **1**.

HRESIMS. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were closely related to those of **3**, except that the signals of methylene protons at δ 2.88 (2H, t, J = 6.0 Hz, H_2-7) and 1.87 (2H, t, J = 6.0 Hz, H_2-8) in **3** were replaced by those of an oxymethine proton at δ 3.79 (1H, dd, J = 7.2, 5.6 Hz, H-8) and methylene protons at δ 3.08 (1H, dd, J = 16.6, 5.6 Hz, H-7a) and 2.79 (1H, dd, J = 16.6, 7.2 Hz, H-7b) in **4**. The HMBC correlations of H-8 with C-3, C-7, C-9, C-10, and C-11 and of H_2-7 with C-2, C-3, C-4, C-8, and C-9 confirmed the location of the OH functionality at C-8. The small J values for H-8 (J = 7.2, 5.6 Hz) indicated that H-8 should be a pseudoequatorial proton. Thus, compound **4** was elucidated as that shown for coumabiocin D.

Compound **5** was isolated as a white, amorphous solid. The molecular formula $C_{31}H_{36}N_2O_{12}$ was determined from the molecular ion peak at m/z 629.2361 ($[M + H]^+$) in its HRESIMS spectrum. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **5** with those of **4** suggested that they were closely related, differing only in the nature of their prenyl substituent. Two broad singlets at δ 4.89 and 4.78 (each 1H, br s, H_2-10) connected to C-10 at δ 111.3 in the HMQC spectrum, suggesting the presence of a 1,1-disubstituted double bond in **5**. In addition, three mutually coupled protons at δ 4.39 (1H, dd, J = 7.8, 6.2 Hz, H-8), 2.98 (1H, dd, J = 14.2, 6.2 Hz, H-7a), and 2.82 (1H, dd, J = 14.2, 7.8 Hz, H-7b) and one vinylic methyl at δ 1.81 (3H, s, H_3-11) suggested that **5** contained a 2-hydroxy-3-methyl-3-butenyl moiety. The presence of this partial structure was also supported by correlations from H_2-7 to C-2, C-3, C-4, C-8, and C-9 and from H_3-11 to C-8, C-9, and C-10 in the HMBC spectrum. In addition, these correlations strongly supported the 2-hydroxyl-3-methyl-3-butenyl group location at C-3. Thus, compound **5** was assigned as coumabiocin E.

Compound **6**, a white, amorphous solid, had the molecular formula $C_{22}H_{21}NO_7$ ($[M + H]^+$ m/z 412.1376) based on a HRESIMS peak, which was 217 mass units less than that of **5**, suggesting the absence of the noviosyl sugar. This proposal was also supported by the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). The ^{13}C NMR and DEPT spectra indicated the presence of 22 carbons as two methyls, two methylenes, six methines, and 12 quaternary carbons. The ^{1}H NMR data showed signals characteristic of a coumarin framework at δ 7.66 (1H, d, J = 8.6 Hz, H-5′), 6.88 (1H, d, J = 8.6 Hz, H-6′), and 2.26 (3H, s, H_3-11′), a 1,3,4-trisubstituted benzene ring at δ 7.80 (1H, br s, H-2), 7.75 (1H, d, J = 8.6 Hz, H-6), and 6.84 (1H, d, J = 8.6 Hz, H-5), and a 2-hydroxy-3-methyl-3-butenyl moiety at δ 4.88 (1H, br s, H-10a), 4.78 (1H, br s, H-10b), 4.40 (1H, dd, J = 7.8, 5.1 Hz, H-8), 2.98 (1H, dd, J = 14.1, 5.1 Hz, H-7a), 2.82 (1H, dd, J = 14.1, 7.8 Hz, H-7b), and 1.81 (3H, s, H_3-11), similar to that found in coumabiocin E. Total assignments of the ^{1}H and ^{13}C resonances (Tables 1 and 2) of **6** were completed through COSY, HMQC, and HMBC data analysis and comparison with **5**. From the above data, compound **6** was assigned the structure shown and named coumabiocin F.

Compounds **1**−**8** were evaluated for their inhibitory activities against *Streptomyces* 85E in the HFI assay (Table 3). Coumabiocins A−E (**1**−**5**) exhibited significant inhibitory activities against *Streptomyces* 85E and gave 10−15 mm clear zones of inhibition (ZOI) at 20 μg/disk. These compounds showed moderate activities and gave a 10 mm bald zone of inhibition at lower concentrations of 2.5 μg/disk, whereas coumabiocin F (**6**) (without the L-noviose group) was inactive (Table 3). Compounds **7** and **8**, a pair of isomers, showed different potency in the HFI assay. Novobiocin (**7**) exhibited potent inhibitory activity and gave a 20 mm clear ZOI at 20 μg/disk and an 18 mm bald ZOI at 2.5 μg/disk. Isonovobiocin (**8**) displayed weaker activity and gave a 10 mm bald zone of inhibition at 20 μg/disk and was inactive at 5 μg/disk. Coumabiocins A (**1**) and D (**4**), with a hydroxy group on the prenyl unit, exhibited weaker inhibition in the HFI assay, with 13 and 10 mm clear zones at 20 μg/disk versus coumabiocins B (**2**) and C (**3**), with 14 and 15 mm clear zones at 20 μg/disk, respectively. The L-noviose group is essential for activity, as observed in **5** (11 mm clear ZOI at 20 μg/disk) versus **6** (inactive). Modification of the isoprenyl unit reduced activity, as observed in **1**−**5** versus **7**.

Table 3. Biological Activity of Tested Compounds on the Growth and Sporulation of *Streptomyces* 85E[a]

compound	zone of inhibition observed (mm)[a,b,c]			
	20 μg/disk	10 μg/disk	5 μg/disk	2.5 μg/disk
coumabiocin A (**1**)	13C	NA[d]	NA	NA
coumabiocin B (**2**)	14C	12C	10B	NA
coumabiocin C (**3**)	15C	10C	NA	NA
coumabiocin D (**4**)	10C	NA	NA	NA
coumabiocin E (**5**)	11C	NA	NA	NA
coumabiocin F (**6**)	NA	NA	NA	NA
novobiocin (**7**)	20C	18C	15C	18B
isonovobiocin (**8**)	10B	9B	NA	NA

[a] Diameter of disk is 7 mm. Stock solutions were prepared in DMSO. No zone of inhibition was observed with DMSO as a negative control. [b] All compounds were tested at 20 μg/disk. Active compounds were retested at lower concentrations (10−2.5 μg/disk). B indicates bald phenotype. [c] Kinase inhibitory activity of compounds was tested on the growth and sporulation of *Streptomyces* 85E. [d] NA indicates not active.

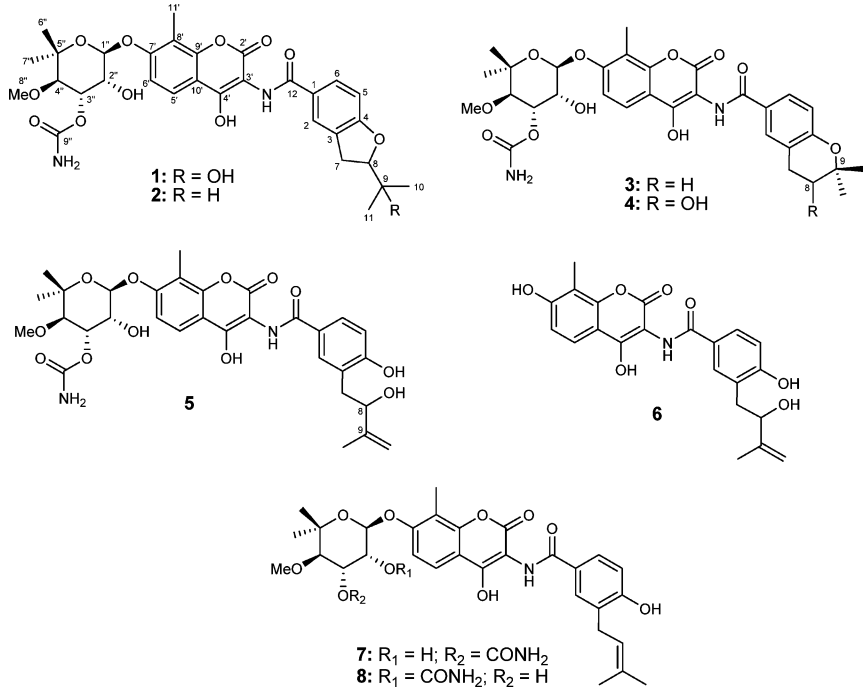
The aminocoumarin antibiotics such as novobiocin, chlorobiocin, and coumermycin A_1, produced by *Streptomyces spheroides*, *S. roseochromogenes*, and *S. rishiriensis*,[8] respectively, are highly potent inhibitors of the bacterial type II topoisomerase DNA gyrase.[9−11] Novobiocin (Albamycin; Upjohn, **7**) is the only aminocoumarin that has been licensed for the treatment of human infection, and its efficacy has been confirmed in several clinical trials.[12,13] Isonovobiocin (**8**), resulting from migration of the carbamoyl unit from O-3″ to O-2″, is reportedly devoid of antibacterial activity.[14] Early investigations on structure−activity relationships among aminocoumarins[15,16] demonstrated that both 3-amino-4,7-dihydroxycoumarin and L-noviose are essential for antibacterial activity and that the substituents attached to these fragments have a significant impact on their bioactivities. Our preliminary findings lend support to previous reports. Structurally modified aminocoumarins have been synthesized and investigated for their bioactivities with respect to that of novobiocin,[17−22] and enhancement of the bioactivity of aminocoumarins while simultaneously improving their toxicological and pharmacological properties appears to be a realizable goal.

Experimental Section

General Experimental Procedures. The specific rotation $[\alpha]_D$ values were determined with an Autopol IV automatic polarimeter. UV spectra were measured on a Shimadzu PharmaSpec-1700 UV−visible spectrophotometer. 1D and 2D NMR spectra were recorded in methanol-d_4 on an INOVA Unity (500 MHz) Varian spectrometer. Mass spectra and high-resolution MS spectra were taken with a BioTOF II ESI mass spectrometer. Reversed-phase HPLC was carried out on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech semipreparative Econosil C_{18} column (10 μm, 10 × 250 mm) with a flow rate of 2.0 mL/min. Column chromatography (CC) was carried out on Merck silica gel 60 (70−230 mesh). Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

Microbial Material and Sample Collection. The organism was isolated from the surface of the lichen *Cladonia gracilis* from Mount Fromme, British Columbia, in November 2002. The strain was isolated using ISP #4 (International *Streptomyces* Project 4, inorganic salts starch agar) media plates supplemented with 50 μg/mL cycloheximide and 20 μg/mL nalidixic acid. The *Streptomyces* L-4-4 is maintained in the laboratory collection at the University of British Columbia and is freely available on request. The strain was identified as a *Streptomyces* species by a 16S rRNA sequence analysis.[23,24] The *Streptomyces* L-4-4 strain was shown to share 98% identity with the strain *Streptomyces caeruleus* via 16S rRNA analysis (1301 base pairs out of 1319). Its sequence has been deposited in GenBank with the accession number GU 980915. The frozen spore stock of *Streptomyces* sp. L-4-4 was stored in 20% glycerol at −78 °C. The vial was thawed, and the spores were spread on an ISP4 plate for complete sporulation. Single colonies were then used to inoculate the seed medium.

Chart 1



Culturing and Harvesting *Streptomyces* sp. L-4-4. The composition of the seed and production medium (in g/L) was as follows: D-glucose (10), glycerol (15), soy peptone (15), NaCl (3), malt extract (5), yeast extract (5), Tween 80 (1), 3-(*N*-morpholino)propanesulfonic acid) [MOPS] (20), and antifoam (3). The medium was prepared with distilled water, and the pH was adjusted to 7.0 prior to sterilization. The medium was dispensed at 50 mL per 250 mL in Belco baffled shaker flasks. A single colony of *Streptomyces* sp. L-4-4 from the ISP4 agar plate was used as inoculum into each flask of media, cultured at 30 °C at 250 rpm for two days. Production medium: An aliquot (1%) from the seed medium was inoculated into the production medium (300 flasks at 500 mL per 2 L in Belco baffled shaker flasks). The composition of the seed medium and production medium was the same. The production cultures were incubated at 30 °C at 250 rpm for seven days and were then harvested. The supernatants were filtered and partitioned with *n*-BuOH three times. Both the *n*-BuOH-soluble and aqueous layers were tested in the hyphae formation inhibition assay.

Extraction and Isolation. The fermentation broth (150 L) was centrifuged, and the supernatant was extracted with *n*-BuOH. The *n*-BuOH-soluble partition significantly inhibited the growth of hyphae formation in *Streptomyces* 85E at a concentration of 80 μg/disk with a 25 mm zone of inhibition. This organic partition (62.5 g) of fermented *Streptomyces* sp. L-4-4 was subjected to CC over silica gel (375 g, 230−400 mesh) using $CHCl_3$ and increasing polarity with MeOH to give five fractions (F1−F5). Fraction F3 (28.2 mg) was further separated by CC over silica gel (175 g, 230−400 mesh) eluting with hexane and increasing polarity with acetone and MeOH, respectively, to afford four fractions (F3a−F3d). Fraction F3c (11.94 g) was purified by Sephadex LH-20 (175 g) using CH_2Cl_2−MeOH (1:1) to give eight subfractions (F3c-1−F3c-8). Subfraction F3c-3 (94.4 mg) was subjected to reversed-phase HPLC eluting with MeCN−H_2O−TFA (60:40:0.05) to give **7** (7.6 mg, t_R 46.6 min) and **8** (9.0 mg, t_R 50.4 min). Subfraction F3c-5 (7.0 mg) was purified by preparative TLC eluting with MeOH−$CHCl_3$ (5:95) to give **1** (1.6 mg) and **4** (2.3 mg). Subfraction F3c-6 (9.3 mg) was further purified by RP-HPLC eluting with MeCN−H_2O−TFA (58:42:0.05) to afford **2** (1.8 mg, t_R 72.8 min) and **3** (1.4 mg, t_R 60.9 min). Subfraction F3c-7 (11.5 mg) was separated by RP-HPLC eluting with MeCN−H_2O−TFA (45:55:0.05) to give **5** (1.6 mg, t_R 36.5 min) and **6** (1.0 mg, t_R 31.5 min).

Coumabiocin A (1): white, amorphous solid; $[\alpha]_D^{21}$ −34.8 (*c* 0.023, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.98), 324 (4.01) nm; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 1 and 2; HRESIMS *m*/*z* 629.2327 $[M + H]^+$ (calcd for $C_{31}H_{37}N_2O_{12}$, 629.2346).

Coumabiocin B (2): white, amorphous solid; $[\alpha]_D^{21}$ −46.5 (*c* 0.027, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.00), 320 (4.02) nm; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 1 and 2; HRESIMS *m*/*z* 613.2415 $[M + H]^+$ (calcd for $C_{31}H_{37}N_2O_{11}$, 613.2397).

Coumabiocin C (3): white, amorphous solid; $[\alpha]_D^{21}$ −47.7 (*c* 0.080, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.89), 324 (3.76) nm; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 1 and 2; HRESIMS *m*/*z* 613.2394 $[M + H]^+$ (calcd for $C_{31}H_{37}N_2O_{11}$, 613.2397).

Coumabiocin D (4): white, amorphous solid; $[\alpha]_D^{21}$ −54.1 (*c* 0.037, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.15), 325 (4.10) nm; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 1 and 2; HRESIMS *m*/*z* 629.2349 $[M + H]^+$ (calcd for $C_{31}H_{37}N_2O_{12}$, 629.2346).

Coumabiocin E (5): white, amorphous solid; $[\alpha]_D^{21}$ −29.4 (*c* 0.017, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.10), 322 (4.09) nm; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 1 and 2; HRESIMS *m*/*z* 629.2361 $[M + H]^+$ (calcd for $C_{31}H_{37}N_2O_{12}$, 629.2346).

Coumabiocin F (6): white, amorphous solid; $[\alpha]_D^{21}$ +3.2 (*c* 0.027, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.21), 322 (4.16) nm; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 1 and 2; HRESIMS *m*/*z* 412.1376 $[M + H]^+$ (calcd for $C_{22}H_{22}NO_7$, 412.1396).

Hyphae Formation Inhibition (HFI) Assay. The inhibition of hyphae formation assay in *Streptomyces* 85E was performed on purified isolates as described previously.[3] The mycelia fragments of *Streptomyces* were spread on minimal medium ISP4 agar plates to generate a bacterial lawn. Compounds of known concentration dissolved in MeOH were dispensed onto disks in 20 μL aliquots. The air-dried disks were applied directly onto the plates and incubated at 30 °C. After 30 h of growth, the results are identified by a clear zone of inhibition and/or bald phenotype around the disk. The sporulation inhibitor surfactin was used as a positive control, and a solvent was employed as a negative control. An inhibition zone greater than 8 mm is considered active. Subfractions were tested at 80 μg/disk on 7 mm filter disks. Active compounds were tested at lower concentrations (20, 10, 5, 2.5 μg/disk). The assays were performed in duplicate.

Acknowledgment. We are grateful to T. Taylor for lichen collection and identification, and H. Wang and I. G. Villanueva for strain isolation and identification by 16S rRNA sequencing. We would like to thank D. Horgen (Hawai'i Pacific University) for assistance with 300 MHz

NMR measurements, and P. Williams and J. Dai (University of Hawai'i Manoa, Department of Chemistry) for assistance with mass spectrometry. The Undergraduate Research Award from the American Society of Pharmacognosy (to N. Vidor) is gratefully acknowledged. Financial support was obtained from NIH-INBRE and start-up funds from the College of Pharmacy.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP900843B