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Subinhibitory Concentrations of Antibiotics Induce Phenazine Production in a Marine *Streptomyces* sp.

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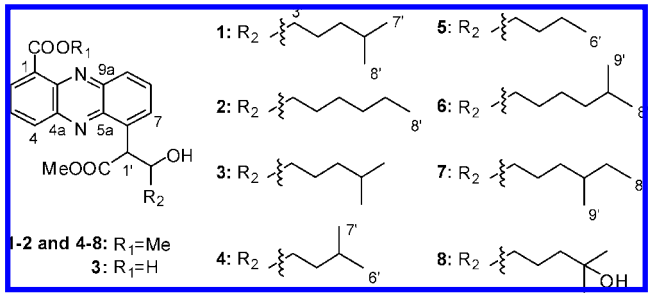
Subinhibitory concentrations of antibiotics were found to enhance and modulate the production of new phenazines, streptophenazines A−H, in a marine *Streptomyces* isolate. The streptophenazines differ in length and substitution of an alkyl chain. The pattern of metabolites formed depends on the antibiotic used. In the presence of tetracycline, streptophenazines F and G were induced and the production of streptophenazines A−D was increased. When using bacitracin, mainly streptophenazine H was produced. Streptophenazines C and H showed moderate activity against *Bacillus subtilis*, while streptophenazine C was also active against *Staphylococcus lentus*.

Recent studies showed that antibiotics at subinhibitory concentrations cause considerable transcriptional changes in various bacteria (for review see ref 1). Investigations on human pathogens and opportunistic pathogenic bacteria[1] revealed that low antibiotic concentrations do not always suppress bacterial development. On the contrary, such low concentrations may lead to the augmentation of some adaptive characteristics.[2–4] Subinhibitory concentrations of tetracycline, for example, increased the cytotoxicity of *P. aeruginosa*,[3] while low amounts of aminoglycoside antibiotics induced biofilm formation in *Pseudomonas aeruginosa* and *Escherichia coli*.[4] Growing evidence supports the idea that antibiotics are hormetic compounds,[5] which act as "chemical weapons" suppressing bacterial growth at higher concentrations, but at subinhibitory concentrations induce different responses that may have relevance for environmental interactions.[1,3] Taking into account the specificity of bacterial responses to different antibiotics, some authors raised the opinion that they act at low doses as signaling molecules.[3]

In a study aimed at revealing if secondary metabolite production of bacteria is affected by subinhibitory antibiotic concentrations, we treated actinomycete strains showing no significant substance peaks in their HPLC-MS profile with various antibiotics. The most interesting results were obtained with a sponge-derived marine *Streptomyces* isolate.



Results and Discussion

The marine *Streptomyces* sp. strain HB202 produced varying amounts of new phenazines with molecular masses 424 (**1**, **2**), 410 (**3**, **4**) and 440 (**8**) and traces of **6** and **7** with a molecular weight of 438, if cultivated under different conditions (Figure 1, Supporting Information Figure S1).[6] Treatment of the cultures with subinhibitory concentrations of tetracycline and bacitracin led to a modulation of the phenazine profiles (Figure 1). Accordingly, tetracycline (1.5 μg/mL) not only caused a 2.8-fold increase in the total phenazine amounts in TSB10 medium, compared to the untreated culture, but also led to 10.7-, 10.7-, and 2.6-fold enhancements of the relative concentrations of **1/2**, **6/7**, and **3**, respectively. Besides that, an 80% inhibition of the production of **8** was detected. The pulse feeding with 0.1 + 0.1 μg/mL bacitracin in TSB10 medium led to a 2.2-fold increment for **8** compared to the untreated culture and to an inhibition of other phenazines.

The fermentations of *Streptomyces* sp. HB202, containing tetracycline or bacitracin, were scaled up to 7.5 or 3 L, respectively. After extraction with ethyl acetate, the crude extracts were separated using FCPC and/or preparative HPLC to yield the new compounds **1**–**8**, named streptophenazines A−H.

Streptophenazine A (**1**) was isolated as an optically active solid, and using HRESIMS in combination with ^{13}C NMR data, the molecular formula $C_{24}H_{28}N_2O_5$ was established for this compound. Resonances for aromatic protons, two methoxyl groups, aliphatic protons, and two methyl groups were apparent in the ^{1}H NMR spectrum, along with resonances for deshielded methine protons (δ 6.15 and 3.27). The specific UV spectrum (λ_{max} 215, 252, 350 (sh), 363 (sh), 367), the appearance of aromatic signals at δ 8.44–7.90, and the presence of four nitrogen-bearing quaternary carbons (δ 143.0, 142.73, 142.66, 141.7) unambiguously disclosed that **1** was a phenazine. The coupling pattern of aromatic protons pointed to a 1,6- or 1,9-disubstituted phenazine skeleton. One of the substituents was identified to be a carbomethoxyl group based on the observed long-range H,C correlations between the methoxyl group at δ 4.08 and a carbonyl signal at δ 168.8, which in turn was correlated to the aromatic proton at δ 8.28 (H-2). The HMBC experiments revealed the second residue to be linked to the phenazine core through a methine group (1′), bearing a carbomethoxyl substituent. Correlations in the COSY and HMBC spectra showed that the 1′-methine was adjoining an oxymethine group, which in turn was linked to a diastereotopic methylene group, followed by two consecutive methylene groups connected to an isopropyl group. Hence, a methyl ester of a 3-hydroxy-7-methyloctanoyl moiety was identified. The NOE correlations between H-9 (δ 8.25) and the methoxyl group at the C-1 substituent (δ 4.08) and between H-4 (δ 8.44) and H-1′ (δ 6.15) established that **1** was a 1,6-disubstituted phenazine.

Streptophenazine B (**2**) was an isomer of streptophenazine A (**1**). This was apparent from the similar mass of the pseudomolecular ion and almost identical ^{1}H NMR spectra with the only difference being the appearance of a signal for only one methyl group (δ 0.76, t, J = 7.3 MHz), explained easily by the presence of an unbranched alkyl chain for C-3′−C-8′. Streptophenazine B was thus identified to have the structure **2**.

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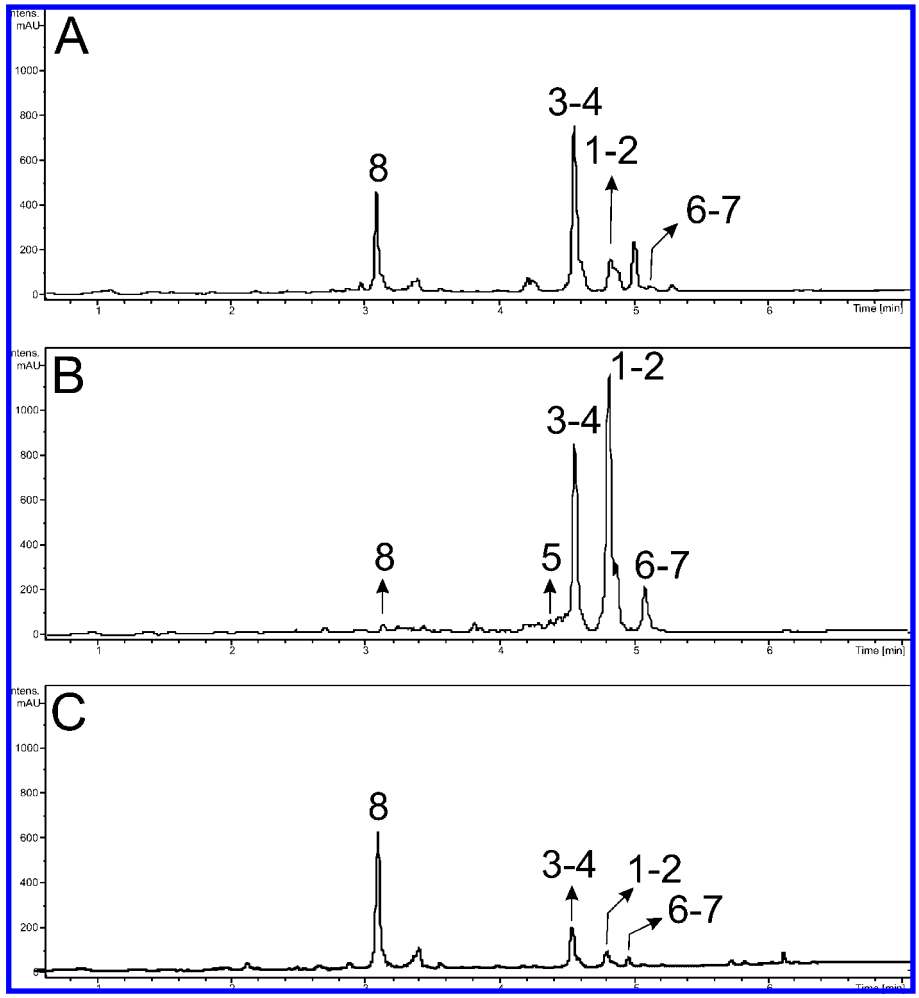

Figure 1. HPLC chromatogram at 254 nm of HB202 cultures grown for 7 days, at 28 °C under shaking conditions. (A) Untreated HB202 culture in TSB10 medium.[6] (B) HB202 culture in TSB10 medium, supplied with 1.5 μg/mL tetracycline. (C) HB202 culture in TSB10 medium, supplied with 0.1 + 0.1 μg/mL bacitracin.

Streptophenazines D (**4**) and E (**5**), which were isolated as minor compounds, were identified as homologues of streptophenazine A (**1**) and streptophenazine B (**2**), respectively, with alkyl chains one methylene group shorter than in **1** for the structure of **4** (C-3′−C-7′) and two methylenes less than in **2** for the structure of **5** (C-3′−C-6′).

Likewise, streptophenazine F (**6**) was found to be a homologue of **1** with a methylene unit longer alkyl chain (C-3′−C-9′). For streptophenazine G (**7**), an isomer of **6**, the presence of a *sec*-butyl end group was found, taking into account the coupling pattern of the methyl groups (δ 0.72, t, J = 7.4 MHz, δ 0.71, d, J = 6.6 MHz) and the distinctive long-range H,C correlations in the HMBC experiment (Supporting Information, Table 7).

Streptophenazine C (**3**) was identified to be desmethoxy-streptophenazine A (**1**), based on the observations of a ^{1}H NMR spectrum similar to that of **1** with signals for only one *O*-methyl group and a pseudomolecular ion (HRESIMS) with 14 mass units less than that of **1**. The HMBC data showed that the C-1 substituent was an unesterified carboxyl group. As further structural proof, streptophenazine A (**1**) was converted by mild alkaline hydrolysis to a compound with the same HPLC retention time and molecular mass as **3**.

A molecular mass 16 Da higher than that of **1** was established for **8** by HRESIMS. The ^{1}H NMR spectra of both compounds were almost identical, with the only obvious difference being that the signals for the methyl groups of **8** appeared as singlets, instead of doublets as in **1**. The long-range H,C correlations from the methyl groups to an oxygen-bearing carbon (δ 71.0) ascertained the presence of a hydroxyl group at C-6′ in the alkyl chain of **8**. Thus, the structure of streptophenazine H was identified to be **8**.

In the fermentations treated with tetracycline, the known compound phenazine-1,6-dicarboxylic acid dimethyl ester[7] was isolated as a minor compound. This compound could be derived from phenazine-1,6-dicarboxylic acid, a common biosynthetic intermediate for most of the phenazines.[8]

The configurations of the stereocenters in the side chains of streptophenaines A−H (**1**–**8**) remain to be elucidated. Preliminary experiments encountered difficulties because of the identical ^{1}H NMR chemical shifts of the Mosher derivatives.

Some of these new streptophenazines exhibited weak to moderate activity against *Bacillus subtilis* (**1**: 46.9 μg/mL, **2**: not active, **3**: 15.6 μg/mL, **4**, **5**: 62.5 μg/mL, **6**, **7**: not active, **8**: 15.6 μg/mL) and *Staphylococcus lentus* (**1**, **2**, **6**, **7**: 62.5 μg/mL, **3**: 46.9 μg/mL). They were inactive against Gram-negative bacteria and *Candida glabrata*.

In cultures supplied with subinhibitory concentrations of antibiotics strain HB202 produced eight phenazines differing in the length and substitution of the alkyl chain. Streptophenazines A−D (**1**−**4**) were formed in moderate amounts in some media without antibiotics (see Experimental Section, Figure 1; Supporting Information, Figure S1),[6] but application of subinhibitory concentrations of tetracycline led to a significant increase of their concentrations (Figure 1, Supporting Information: Figure S1). Tetracycline also induced the formation of significant amounts of streptophenazines F (**6**) and G

(**7**), found in all antibiotic-free media only in traces. Moderate enhancement of the concentrations of streptophenazine H (**8**) was observed after pulse application of bacitracin only in TSB10 medium.

Furthermore, studies on the time–concentration profiles of untreated HB202 cultures and fermentations supplemented with tetracycline and bacitracin indicated that streptophenazines were produced earlier and in higher concentrations in the antibiotic-stimulated cultures, further corroborating that the observed changes in the streptophenazine profiles were caused by the antibiotics.[9]

The experimental data show that subinhibitory concentrations of antibiotics exhibited a selective influence on the amounts and the pattern of streptophenazines formed. The supplement of tetracycline to the fermentations of strain HB202 increased the total phenazine amounts and encouraged the biosynthetic steps leading to methylation of the C-1 carboxyl group (**1**) and to the elongation of the alkyl substituent at C-6 (**2**, **6**, **7**) and blocked those leading to the hydroxylation of C-6′ (**8**). An enhancement of the reactions resulting in a hydroxyl substituent at C-6′ (**8**) and inhibition of other biosynthetic steps was observed during pulse supplement with bacitracin. The induction and modulation of the biosynthesis of the antibacterial streptophenazines suggests that subinhibitory concentrations of antibiotics might cause a defensive response in bacteria.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were registered on a Perkin-Elmer UV/vis spectrophotometer Lambda 11. NMR spectra were recorded on a Bruker AV600 spectrometer (600 and 150 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 3.31 and δ_C 49.0 ppm for MeOH-d_4). Fast centrifugal partitioning chromatography (FCPC) was performed on a system equipped with a 200 mL rotor (Kromaton). High-resolution mass spectra were acquired on a FT-ICR spectrometer (7 T Apex-Qe, Brucker) with positive electrospray ionization. HPLC analyses were performed using a C18 column (Phenomenex Onyx Monolithic C18, 100 × 3.00 mm) applying a H_2O/MeCN gradient with 0.1% HCOOH added to both solvents. For MS detection the HPLC was coupled to an ESI-ion trap system (Bruker Daltonics Esquire 4000).

Isolation and Identification of *Streptomyces* sp. HB202. The isolate HB202 was obtained from the marine sponge *Halichondria panicea* from the Baltic Sea (Germany). A specimen of approximately 1 g was macerated in sterilized sea water, and the suspension was plated on a semisynthetic polycarbon (HSPC) medium[10] with 1.5% NaCl. The incubation was performed in the dark at 22 °C for 15 days. Colonies were streaked several times on TSB10 medium (3 g of BD trypticase soy broth (TSB), 10 g of NaCl, 1 L of water, pH 7.2) in order to obtain a pure culture.

DNA extraction, amplification, and sequencing of 16S rDNA were performed using standard protocols.[11,12] The comparison of the 16S rDNA sequence (1364 nucleotides) of the sponge-derived isolate HB202 with sequences in the EMBL nucleotide database available online at the European Bioinformatics Institute homepage and of RDP-II Project homepage[13] revealed that the strain was affiliated with the genus *Streptomyces*. The most closely related type strain was *S. griseus* ATCC 51928^T (GenBank/EMBL/DDBJ acc. no. AF112160) with a sequence similarity of 99.8%.

Study on the Influence of Different Media on the Production of Streptophenazines. *Streptomyces* sp. HB202 was grown in multiple 100 mL Erlenmeyer flasks each containing 10 mL of seven different media for 7 days at 28 °C under shaking conditions (120 rpm). The following media were used: TSB10, modified TSB (TSBm, 3 g of BD trypticase soy broth, 10 g of NaCl, 2 g of $CaCO_3$, 1 L of water, pH 7.2), GYM1 (4 g of glucose, 4 g of yeast extract, 4 g of malt extract, 1 L of water, pH 7.2), GYM2 (10 g of glucose, 4 g of yeast extract, 4 g of malt extract, 1 L of water, pH 7.2), M1 (5 g of peptone, 3 g of meat extract, 1 L of water, pH 7.2), LB (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, 1 L of water, pH 7.2), and MB (37.4 g of Difco marine broth, 1 L of water, pH 7.2). Seed cultures (10 mL of TSB10 in 100 mL Erlenmeyer flasks) were grown for 5 days prior to transfer. Each culture was inoculated separately, in duplicate, with 200 μL of the strain seed culture. Additionally, fermentations of strain HB202 in different media were supplied separately, in duplicate, with a sterile-filtered solution of tetracycline (15 μg in 100 μL of MeOH at the time of inoculation; 1.5 μg/mL final concentration in the culture) or by feeding with 1 μg of bacitracin (in 100 μL of MeOH) at the time of inoculation and another 1 μg (in 100 μL of MeOH) on the second day of fermentation (0.2 μg/mL final concentration in the culture). The stock solutions of antibiotics were prepared in MeOH. After 7 days all cultures were extracted with 10 mL of EtOAc (×2). After evaporation of the solvent the extracts were redissolved in 200 μL of MeOH. Aliquots of 15 μL were analyzed by HPLC-MS. Varying amounts of streptophenazines were produced in TSB10, GYM1, M1, and LB without supplement of antibiotics; no streptophenazines were biosynthesized in untreated HB202 cultures on TSBm, GYM2, and MB (Supporting Information, Figure S1). Addition of tetracycline led to an increase of the streptophenazine concentrations in all media except for MB (Supporting Information, Figure S1). Addition of bacitracin led to the enhancement of streptophenazine H (**8**) quantities only on TSB10.

Minimal Inhibition Concentrations (MIC) for Tetracycline and Bacitracin. For this experiment *Streptomyces* sp. HB202 was grown in 2 mL of TSB10 medium in a 20 mL vial (27 × 50 mm) for 7 days at 28 °C under shaking conditions (120 rpm). Seed cultures were prepared as described above. Each vial was inoculated with 40 μL of the strain seed culture and 20 μL of MeOH solution of different concentrations (0–60 μg/mL) of tetracycline or bacitracin (in quadruplicate). MICs of 10 and 25 μg/mL were determined for tetracycline and bacitracin, respectively.

Fermentation. For chemical investigation the strain HB202 was grown for 7–15 days at 28 °C under shaking conditions (120 rpm). For the tetracycline-stimulation experiments (25 × 300 mL of GYM1) each culture was supplied with 450 μg of tetracycline dissolved in 3 mL of MeOH (1.5 μg/mL final concentration) at the time of inoculation. In the bacitracin-treated cultures (10 × 300 mL of TSB10), the antibiotic was added twice (2 × 30 μg of bacitracin dissolved in 3 mL of MeOH, 0.2 μg/mL final concentration), first at the time of inoculation and then on the second day of fermentation.

Purification of Streptophenazines A−H (1–8). The fermentation broth was centrifuged (4700*g* for 20 min), and the pellet was recovered. The culture broth (A, 7.5 L for tetracycline-treated cultures; B, 3 L for bacitracin-stimulated cultures) was extracted with equal volumes of EtOAc (4 × 7.5 and 4 × 3 L, respectively). The extracts were dried to yield the crude extract (A, 702 mg; B, 103 mg). In addition, the cells were macerated with MeOH (3 × 20 mL) and aliquots analyzed with HPLC-MS. Only in the cell extract of tetracycline-treated cultures was the presence of streptophenazines observed.

The crude extract A (702 mg) was combined with the corresponding cell extract (47 mg) and subjected to FCPC, using the two-phase solvent system *n*-heptane−EtOAc−MeOH−H_2O (5.5:4.5:5.5:4.5). The upper phase served as the stationary phase (flow rate 7 mL/min, rotation speed 1400 rpm). After 84 min the stationary phase was flushed out backward with MeOH. Fractions containing streptophenazines were further purified by HPLC using a C18 column (Phenomenex Luna C18(2), 250 × 4.60 mm, 5 μm, eluents: H_2O (A), MeCN (B); flow 2 mL/min; UV detection at 254 nm). FCPC fractions collected at 33–42 min were purified using gradient conditions (0 min 60% B, 16 min 90% B) to yield **5** (6.99–7.5 min, 0.2 mg), **4** (7.95–8.76, 0.3 mg), and **3** (9.57–10.66, 0.9 mg). FCPC fractions eluted at 51–69 min were chromatographed at isocratic conditions using 60% B to give **1** (9.49–10.44, 5.1 mg) and **2** (10.51–11.51, 3.6 mg). Fractions from the FCPC run collected at 75–81 min were further purified at isocratic conditions (48% B), yielding **6** (49.77–52.43, 7.8 mg) and **7** (46.54–49.55, 3 mg). The FCPC fraction eluted at 132 min was purified by gradient elution (0 min 10% B, 30 min 100% B) to give phenazine-1,6-dicarboxylic acid dimethyl ester[7] (18.46–18.80, 0.1 mg).

Extract B (103 mg) was subjected to preparative HPLC (Phenomenex Luna C18 (2), 21.2 × 250 mm, 5 μm; eluents: H_2O (A), MeCN (B); gradient 0 min 30% B, 20 min 90% B; flow 20 mL/min; UV detection at 254 nm). Streptophenazines H (**8**, 1.0 mg), C (**3**, ≤0.1 mg), and A (**1**, ≤0.1 mg) were eluted at 10.5–11.4, 17.0–18.5, and 19.3–21 min, respectively.

Streptophenazine A (1): yellow, amorphous solid, $[\alpha]^{20}_D$ −50 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.10), 252 (4.98), 350 (sh)

(4.10), 363 (sh) (4.26), 367 (4.29); for 1D and 2D NMR, see SI Table 1; HRESIMS *m/z* 425.2063 $[M + H]^+$ (calcd for $C_{24}H_{29}N_2O_5$, 425.2076).

Streptophenazine B (2): yellow, amorphous solid, $[\alpha]^{20}_D$ −45 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.10), 252 (4.98), 351 (sh) (4.10), 364 (sh) (4.26), 368 (4.29); for 1D and 2D NMR, see SI Table 2; HRESIMS *m/z* = 425.2071 $[M + H]^+$ (calcd for $C_{24}H_{29}N_2O_5$, 425.2076).

Streptophenazine C (3): reddish, amorphous solid, $[\alpha]^{20}_D$ −36 (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.10), 252 (4.98), 354 (sh) (4.10), 364 (sh) (4.26), 371 (4.29); for 1D and 2D NMR, see SI Table 3; HRESIMS *m/z* 411.1913 $[M + H]^+$ (calcd for $C_{23}H_{27}N_2O_5$, 411.1920).

Streptophenazine D (4): yellow, amorphous solid, $[\alpha]^{20}_D$ −41 (*c* 0.017, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.12), 252 (4.98), 351 (sh) (4.09), 364 (sh) (4.26), 368 (4.29); for ^{1}H and COSY NMR, see SI Table 4; HRESIMS *m/z* 411.1914 $[M + H]^+$ (calcd for $C_{23}H_{27}N_2O_5$, 411.1920).

Streptophenazine E (5): yellow, amorphous solid, $[\alpha]^{20}_D$ −34 (*c* 0.017, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.12), 252 (4.98), 351 (sh) (4.08), 364 (sh) (4.25), 368 (4.29); for ^{1}H and COSY NMR, see SI Table 5; HRESIMS *m/z* 397.1755 $[M + H]^+$ (calcd for $C_{22}H_{25}N_2O_6$, 397.1763).

Streptophenazine F (6): yellow, amorphous solid, $[\alpha]^{20}_D$ −34 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.10), 252 (4.98), 353 (sh) (4.09), 364 (sh) (4.26), 368 (4.29); for 1D and 2D NMR, see SI Table 6; HRESIMS *m/z* 439.2227 $[M + H]^+$ (calcd for $C_{25}H_{31}N_2O_5$, 439.2233).

Streptophenazine G (7): yellow, amorphous solid, $[\alpha]^{20}_D$ −35 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.10), 252 (4.98), 351 (sh) (4.09), 363 (sh) (4.26), 368 (4.29); for 1D and 2D NMR, see SI Table 7; HRESIMS *m/z* 439.2226 $[M + H]^+$ (calcd for $C_{25}H_{31}N_2O_5$, 439.2233).

Streptophenazine H (8). reddish, amorphous solid, $[\alpha]^{20}_D$ −24 (*c* 0.017, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.12), 252 (4.96), 352 (sh) (4.07), 363 (sh) (4.25), 368 (4.28); for 1D and 2D NMR, see SI Table 8; HRESIMS *m/z* 441.2014 $[M + H]^+$ (calcd for $C_{24}H_{29}N_2O_6$, 441.2026).

Conversion of Streptophenazine A (1) in Streptophenazine C (3). To 0.1 mg of solution of **1** LiOH (500 μL; 0.1 M in MeOH−H_2O (8:2)) was added. This mixture was stirred for 1 h at room temperature and then directly analyzed by HPLC-MS. The main product of the reaction has a retention time and mass spectrum identical with those of streptophenazine C (**3**).

Biological Activities of Streptophenazines. Streptophenazines **1**−**3**, **6**, and **7** were tested against Gram-positive (*Bacillus subtilis, Staphylococcus lentus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas fluorescens*), Gram-negative phytopathogenic bacteria (*Pseudomonas syringae, Xanthomonas campestris, Erwinia amylovora*, *Ralstonia solanacearum*), and a yeast (*Candida glabrata*). The minor compounds streptophenazines **4**, **5**, and **8** were tested only against *B. subtilis*. Bioactivity assays were measured using standard protocols.[14]

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Supporting Information Available: HPLC chromatograms of HB202 fermentations, Tables with 1D and 2D NMR data for **1**–**8**, ^{1}H NMR spectra of **1**–**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Davies, J.; Spiegelman, G. B.; Yim, G. *Curr. Opin. Microbiol.* **2006**, *9*, 445–453.
(2) Goh, E.-B.; Yim, G.; Tsui, W.; McClure, J.; Surette, M.; Davies, J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 17025–17030.
(3) Linares, L. F.; Gustafsson, I.; Baquero, F.; Martinez, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 19484–19489.
(4) Hoffman, L. R.; D'Argenio, D. A.; MacCoss, M. J.; Zhang, Z.; Jones, R. A.; Miller, S. I. *Nature* **2005**, *436*, 1171–1175.
(5) Hormesis is a term used to describe the biological responses to environmental challenges with opposite effect in small and large doses: Calabrese, E.; Baldwin, L. *Hum. Exp. Toxicol.* **2002**, *21*, 91–97.
(6) The presence of streptophenazine 5, isolated as a minor compound from the HB202 fermentations stimulated with tetracycline, could not be unambiguously detected in the untreated cultures.
(7) Roemer, A. *Org. Magn. Reson.* **1982**, *19*, 66–8.
(8) Laursen, J. B.; Nielsen, J. *Chem. Rev.* **2004**, *104*, 1663–1685.
(9) Mitova, M. I.; Wiese, J.; Imhoff, J. F. *Mar. Biotechnol.* 2008, in preparation.
(10) Muscholl-Silberhorn, A.; Thiel, V.; Imhoff, J. F. *Microb. Ecol.* **2008**, *55*, 94–106.
(11) Alexander, B.; Imhoff, J. F. *Int. Microbiol.* **2006**, *9*, 259–266.
(12) Thiel, V.; Neulinger, S. C.; Staufenberger, T.; Schmaljohann, R.; Imhoff, J. F. *FEMS Microbiol. Ecol.* **2007**, *59*, 47–63.
(13) Cole, J. R.; Chai, B.; Farris, R. J.; Wang, Q.; Kulam-Syed-Mohideen, A. S.; McGarrell, D. M.; Bandela, A. M.; Cardenas, E.; Garrity, G. M.; Tiedje, J. M. *Nucleic Acids Res.* **2007**, *35*, D169–D172.
(14) Lang, G.; Wiese, J.; Schmaljohann, R.; Imhoff, J. F. *Tetrahedron* **2007**, *63*, 11844–11849.

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