JOURNAL OF NATURAL PRODUCTS

NOTE

pubs.acs.org/jnp

Farinamycin, a Quinazoline from *Streptomyces griseus*

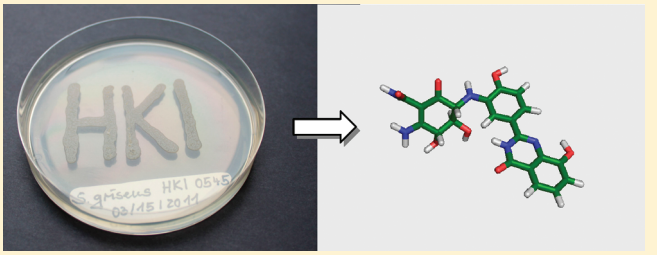
Markus Nett*,† and Christian Hertweck‡,§

†Junior Research Group "Secondary Metabolism of Predatory Bacteria" and ‡Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Beutenbergstrasse 11a, 07745 Jena, Germany

§Friedrich Schiller University, 07737 Jena, Germany

Supporting Information

ABSTRACT: The metabolic potential of a *Streptomyces griseus* strain was investigated under various cultivation conditions. After fermentation in a flour-based medium, a new quinazoline metabolite, farinamycin (**1**), could be isolated from the bacterium, which was previously known only for the production of phenoxazinone antibiotics. The structure of **1** illuminates the biosynthetic versatility of *S. griseus*, which assembles a defined set of building blocks into structurally diverse natural products.



The genus *Streptomyces* is recognized as the most important bacterial source of clinically used therapeutics, including life-saving antibiotics and antifungal, anticancer, and immunosuppressive agents.[1] Genome sequencing projects not only confirmed the metabolic prowess of this bacterial group but also unveiled a huge potential for the discovery of as yet untapped chemical diversity.[2] These observations have spurred a renewed interest in natural product discovery from actinomycetes in general and *Streptomyces* spp. in particular.[3] In the past decade, various strategies have been explored to access the products of the diverse biosynthetic pathways that are encoded on bacterial genomes.[4−6] A rather useful, albeit apparently random approach to induce or enhance the expression of biosynthetic genes is to subject the target organism to altered growth conditions.[7,8] This method relies on the well-known observation that certain environmental factors, i.e., foreign physical and chemical stimuli, trigger metabolic responses, including natural product biosynthesis. Applying this strategy to a *Streptomyces griseus* strain led to the isolation and characterization of a new metabolite, farinamycin (**1**), which likely derives from the condensation of 3-hydroxyanthranilic acid (3-HAA) with 3-amino-4-hydroxybenzamide (3,4-AHBAm) and the linkage of the latter with an epoxyquinone precursor.

The *S. griseus* strain HKI 0545 used in this study was isolated from the plaster of an old building and was previously shown to produce a diverse set of phenoxazinone antibiotics with antiproliferative properties when grown in a soybean/glucose medium.[9,10] Using the published spectroscopic data for dereplication,[9,10] the metabolome of this strain was reinvestigated under various growth conditions. To this end, the strain was cultured on a small scale (50 mL) in different media, and the metabolic profile of each culture was recorded by LC-MS following ethyl acetate extraction. The extract that originated from a farina-based culture revealed the presence of a distinct peak, the UV profile of which did not match a phenoxazinone scaffold. The detected pseudomolecular ion at m/z 452 $[M - H]^-$ appeared promising as a novel compound, and therefore the fermentation was repeated on a 1 L scale. The ensuing culture broth was centrifuged, and the supernatant was extracted with ethyl acetate. A preliminary fractionation was accomplished by flash column chromatography on RP silica gel using increasing concentrations of methanol in water as eluent. Fractions that contained the target ion at m/z 452 $[M - H]^-$ were subsequently pooled and subjected to semipreparative RP-HPLC to give 1.5 mg of pure farinamycin (**1**, Figure 1).

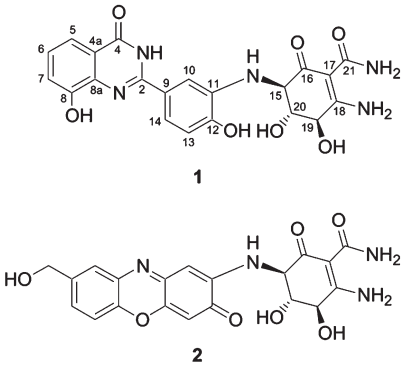


Figure 1. Structures of farinamycin (**1**) and pitucamycin (**2**). The relative configuration is depicted.

The empirical formula of **1** was assigned to be $C_{21}H_{19}O_7N_5$ by HRESIMS, which corresponds to 15 degrees of unsaturation. The IR spectrum showed a broad band at 3274 cm^{-1}, suggesting associated hydroxyl groups. Strong stretching vibrations at 1668

Received: March 22, 2011
Published: September 22, 2011

Table 1. NMR Spectroscopic Data for 1 in Pyridine-d_5

position	δ_C	δ_H, M (J in Hz)	HMBC	NOE
2	154.6			
4	164			
4a	123			
5	117.1	8.12, dd (7.9, 1.5)	4, 7, 8a	6
6	127.2	7.38, t (7.9, 7.8)	4a, 8	5, 7
7	118.8	7.50, dd (7.8, 1.5)	5, 8, 8a	6
8	154.6			
8a	140.2			
9	126.4			
10	114	8.52, d (2.2)	2, 11, 12, 14	15
11	141.2			
12	150.4[a]			
13	114.5	7.23, d (8.1)[b]	9, 11, 12	14
14	119.3	7.73, dd (8.1, 2.2)	2, 10, 12	13
15	66.3	4.06, d (10.6)	11, 16, 19, 20	10, 19
16	192.6			
17	98.8			
18	175.3			
19	73.4	4.62, d (9.5)	18, 20	15
20	77.1	4.20, t (10.6, 9.5)	15, 19	
21	172.7			
18-NH_2		a: 12.12, d (6.8)	17, 19	18-NH_2 (b)
		b: 9.23, d (6.8)	17, 19	18-NH_2 (a)
21-NH_2		a: 10.05, d (4.6)		21-NH_2 (b)
		b: 7.85, d (4.6)	17, 21	21-NH_2 (a)

[a] Deduced from HMBC data. [b] Deduced from a selective NOE experiment due to resonance overlapping with solvent signal.

and 1635 cm^{-1} indicated the presence of carbonyl moieties. In the ^{13}C NMR spectrum of **1** only 20 signals were resolved. Subsequent analyses revealed that the missing resonance was concealed by the solvent signal, and its chemical shift (150.4 ppm, C-1′) had to be deduced from HMBC data (see Supporting Information). Inspection of the proton NMR and NOE spectra allowed the identification of nine nonexchangeable protons, which could be distributed among three discrete spin systems based on first-order multiplet analysis (Table 1). Three resonances in the aromatic region occur as an isolated AMX spectrum (δ_A 7.23, δ_M 7.73, δ_X 8.52, J_{AM} = 8.1 Hz, J_{MX} = 2.2 Hz) characteristic of a 1,2,4-trisubstituted benzene derivative. Likewise, the aromatic protons at δ_H 8.12, 7.50, and 7.38 ppm were assigned to a 1,2,3-trisubstituted benzene. The nonexchangeable protons in the upper field (δ_H 4.62, 4.20, and 4.06 ppm) constitute a spin system of three adjacent sp^3-hybridized methine groups. The remaining four ^1H NMR resonances that disappeared upon treatment with D_2O were identified as the protons of two NH_2 groups. Geminal couplings were detected between the protons δ_H 12.12 and 9.23 of 18-NH_2 and the protons δ_H 10.05 and 7.85 of 21-NH_2, respectively. By means of HSQC the C-bound protons were assigned to their adjacent carbon atoms. HMBC data revealed that the NH_2 groups as well as the aforementioned sp^3-hybridized methine groups are part of a cyclohexenone substructure that was previously described in the phenoxazinone pitucamycin (**2**, Figure 1).[9] A key $^1H-^{13}C$ long-range correlation from H-15 to C-11 allowed the linkage of this moiety with the 1,2,4-trisubstituted benzene ring. The positioning of the

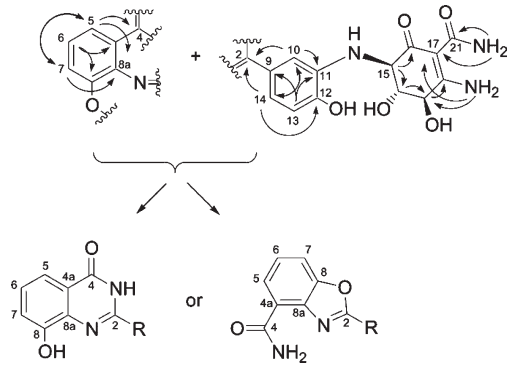
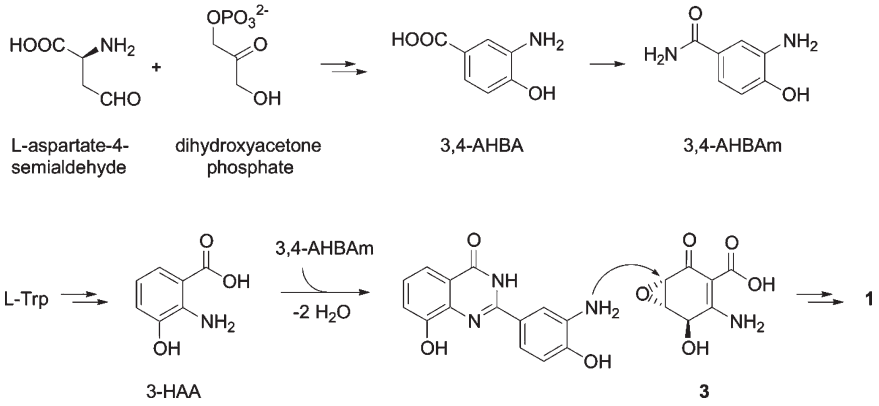


Figure 2. Key HMBC correlations in **1** established two fragments that could be fused to either a quinazolinone or a benzoxazole carboxamide framework (R = 3-(4-amino-3-carbamoyl-5,6-dihydroxy-2-oxo-cyclohex-3-enylamino)-4-hydroxyphenyl). The observed NMR chemical shifts in **1** are consistent only with a quinazolinone substructure.

protons on the aromatic ring was deduced from HMBC correlations (Figure 2). The upfield-shifted resonances of C-10 and C-13 suggested the presence of electron-donating substituents at C-11 and C-12, respectively, and the chemical shifts of the latter supported the assignment of an amine at C-11 (δ_C 141.2) and of a hydroxyl function at C-12 (δ_C 150.4). The chemical shift of C-9 (δ_C 126.4) excluded the linkage with a heteroatom, while HMBC correlations from H-10 and H-14 to a carbon resonating at δ_C 154.6 established C-2 as the C-9 substituent. The HMBC connectivities of the protons in the remaining 1,2,3-trisubstituted benzene moiety, together with the chemical shifts of C-8 (δ_C 154.6) and C-8a (δ_C 140.2), revealed the presence of O- and N-bound substituents at C-8 and C-8a, respectively, and allocated C-4 at position 4a. In consideration of the HRESIMS data, two alternatives are conceivable to link the deduced fragments, giving rise to either a benzoxazole carboxamide or a quinazolinone scaffold (Figure 2). The former proposal was excluded for two main reasons: (i) no additional N-bound protons corresponding to a primary amide function were detected in the proton NMR spectra of **1**, and (ii) the chemical shifts of C-2 (δ_C 154.6) and C-5 (δ_C 117.1) were inconsistent with values reported for benzoxazole antibiotics exhibiting the same substitution pattern.[11−14] Furthermore, the respective ^{13}C NMR chemical shifts were in perfect agreement with those of 8-hydroxyquinazolin-4-ones.[15,16] After switching to dimethylsulfoxide-d_6 as a solvent, all exchangeable protons of **1** were detected (except for the NH group at position 3),[17] and their $^1H-^{13}C$ long-range correlations confirmed the proposed quinazoline framework, thus establishing the planar structure of **1**. The relative configuration of the cyclohexenone moiety in farinamycin (**1**) corresponds to that in pitucamycin (**2**).[9] The large coupling constant between H-15 and H-20 (J = 10.6 Hz) indicated the axial position of both protons. The *anti* configuration of H-19 and H-20 was also evident from the corresponding coupling constant (J = 9.5 Hz). Moreover, a strong NOESY correlation was observed between the protons H-15 and H-19, corroborating their *syn* configuration.

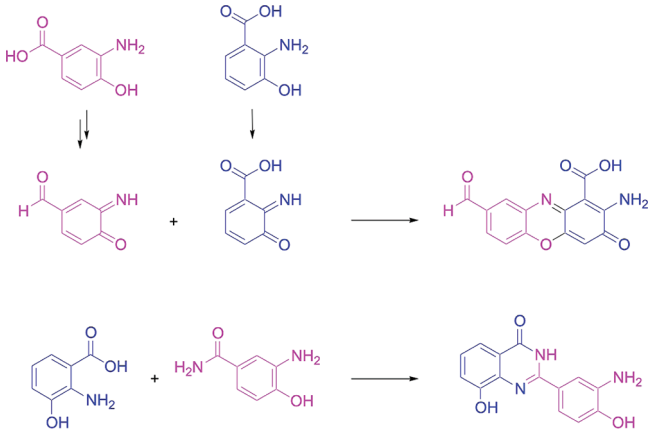
The antimicrobial activities of farinamycin (**1**) were evaluated in an agar diffusion assay against a series of Gram-positive and Gram-negative bacteria as well as fungi; however, the metabolite turned out to be inactive at concentrations ≤ 100 μg/mL. **1** also lacked antiproliferative or cytotoxic effects toward HUVEC, K-562, and HeLa cells at concentrations ≤ 50 μg/mL.

Scheme 1. Proposed Assembly of Farinamycin (1)[a]



[a] Model for the formation of **1** through (a) Niementowski-type condensation of 3-hydroxyanthranilic acid (3-HAA) with 3-amino-4-hydroxybenzamide (3,4-AHBAm) and (b) aminolytic epoxide cleavage.

Scheme 2. Biosynthetic Divergence in *S. griseus* HKI 0545[a]



[a] Phenoxazinones and quinazolines are proposed to be synthesized from 3-HAA- and 3,4-AHBA-derived precursors.

The structure of farinamycin suggests an assembly from three distinct precursors, namely, 3-HAA, 3,4-AHBAm, and enaminomycin C (**3**, Scheme 1).[18,19] The quinazoline skeleton of **1** can be readily rationalized as the result of a Niementowski-type reaction from the former two building blocks,[20] while the incorporation of the epoxyquinone enaminomycin C may involve a nucleophilic oxirane ring-opening, as proposed in pitucamycin biosynthesis.[9] The observed *anti* configuration between H-15 and H-20 would support this hypothesis. A structural comparison of the quinazoline **1** with previously isolated phenoxazinones clearly reveals that both classes of natural products share the same building blocks (Scheme 2).[21,22] It remains to be determined whether the divergent annulation of the common precursors in **1** is enzyme-mediated or due to prevailing reaction kinetics impacted by the pH of the fermentation medium.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation was measured using a 0.5 dm cuvette with a JASCO P-1020 polarimeter at 25 °C. UV spectra were recorded on a Varian UV−visible Cary spectrophotometer. IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. NMR spectra were measured at 300 K on Bruker Avance III 600 and Bruker Avance III 500 spectrometers with pyridine-d_5 or dimethylsulfoxide-d_6 as solvent and internal standard. ESIMS data were obtained with a triple quadrupole mass spectrometer (Quattro; VG Biotech, Altrincham, Cheshire, UK). Analytical HPLC was performed on an Agilent 1100 Series LC/MSD trap. Preparative HPLC was conducted on a Shimadzu HPLC system (LC-20AT, SPD-M20A).

Isolation of Farinamycin. Cultivation was performed in a 5 L Erlenmeyer flask, containing 1 L of a farina-based medium (4% farina supplemented with 5 mL of trace element solution: $ZnCl_2$ 40 mg/L, $FeSO_4 \cdot 7H_2O$ 200 mg/L, $CuCl_2 \cdot 7H_2O$ 10 mg/L, $MnCl_2 \cdot 4H_2O$ 10 mg/L, H_3BO_3 5 mg/L, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 10 mg/L, HCl conc. 2 mL). The flask was inoculated with a 100 mL preculture grown in the same medium and shaken on a rotary shaker (140 rpm) at 28 °C for four days. At the end of the cultivation, the bacterial cells were removed from the culture broth by centrifugation. The supernatant was extracted with ethyl acetate (3 × 1 L). After evaporation of the solvent, the residue was resuspended in 20% aqueous methanol and subjected to flash column chromatography over Polygoprep 60-50 C18 (Macherey-Nagel) using a gradient of MeOH/H_2O as eluent. Farinamycin-containing fractions were combined and further purified by RP-HPLC (column: VP 250/10 Nucleodur C18 HTec, 5 μm (Macherey-Nagel); eluent: 10% acetonitrile for 5 min, 10% → 40% within 30 min; flow rate 2.0 mL/min; detection: UV absorption) to yield 1.5 mg of **1**.

Farinamycin (1): white powder; $[\alpha]^{24}_D$ −43.9 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 343 (3.94), 246 (4.35) nm; IR (film) ν_{max} 3274, 1668, 1635, 1583, 1533, 1469, 1380, 1288, 1228, 1109, 1052, 753 cm^{-1}; ^{1}H and ^{13}C NMR, Table 1; HRESIMS *m/z* 452.1224 [M − H]$^-$, calcd 452.1212 for $C_{21}H_{18}O_7N_5$.

Biological Assays. Antimicrobial activities of **1** were determined in a primary screen against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (SG 511), *Escherichia coli* (SG 458), *Pseudomonas aeruginosa* (K 799/61), *Mycobacterium vaccae* (IMET 10670), *Sporobolomyces salmonicolor* (SBUG 549), *Candida albicans* (BMSY 212), and *Penicillium notatum* (JP 36). Antiproliferative and cytotoxic effects were evaluated against cell lines K-562 (human chronic myeloid cells, DSM ACC 10), HUVEC (vascular endothelium cells, ATCC CRL-1730), and HeLa (human cervix carcinoma, DSM ACC 57), respectively.[10]

ASSOCIATED CONTENT

Supporting Information. NMR spectra of compound **1** and HPLC profiles of *S. griseus* strain HKI 0545. This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/np2002513 |*J. Nat. Prod.* 2011, 74, 2265–2268

AUTHOR INFORMATION

Corresponding Author
*Tel: + 49 3641 5321297. Fax: +49 3641 5320811. E-mail: Markus.Nett@hki-jena.de.

ACKNOWLEDGMENT

This work was generously supported by a grant from the Bundesministerium für Bildung und Forschung (0315591A to M.N.). We thank A. Perner, H. Heinecke, and U. Valentin for recording MS, UV, and IR spectroscopic data and P. B. Gomes, J. Gressler, M. F. Kreutzer, and N. Domin for technical support. C. Weigel and H.-M. Dahse are gratefully acknowledged for conducting the agar diffusion assay and cytotoxicity assays, respectively.

REFERENCES

(1) Hopwood, D. A. *Streptomyces in Nature and Medicine*; Oxford University Press, 2007.
(2) Nett, M.; Ikeda, H.; Moore, B. S. *Nat. Prod. Rep.* **2009**, *26*, 1362–1384.
(3) Baltz, R. H. *Curr. Opin. Pharmacol.* **2008**, *8*, 557–563.
(4) Zerikly, M.; Challis, G. L. *ChemBioChem* **2009**, *10*, 625–633.
(5) Gross, H. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 207–219.
(6) McAlpine, J. B. *J. Nat. Prod.* **2009**, *72*, 566–572.
(7) Scherlach, K.; Hertweck, C. *Org. Biomol. Chem.* **2009**, *7*, 1753–1760.
(8) Bode, H. B.; Bethe, B.; Höfs, R.; Zeeck, A. *ChemBioChem* **2002**, *3*, 619–627.
(9) Gomes, P. B.; Nett, M.; Dahse, H.-M.; Hertweck, C. *J. Nat. Prod.* **2010**, *73*, 1461–1464.
(10) Gomes, P. B.; Nett, M.; Dahse, H.-M.; Sattler, I.; Martin, K.; Hertweck, C. *Eur. J. Org. Chem.* **2010**, 231–235.
(11) Hohmann, C.; Schneider, K.; Bruntner, C.; Irran, E.; Nicholson, G.; Bull, A. T.; Jones, A. L.; Brown, R.; Stach, J. E. M.; Goodfellow, M.; Beil, W.; Krämer, M.; Imhoff, J. F.; Süssmuth, R. D.; Fiedler, H.-P. *J. Antibiot.* **2009**, *62*, 99–104.
(12) Sommer, P. S. M.; Almeida, R. C.; Schneider, K.; Beil, W.; Süssmuth, R. D.; Fiedler, H. P. *J. Antibiot.* **2008**, *61*, 683–686.
(13) Sato, S.; Kajiura, T.; Noguchi, M.; Takehana, K.; Kobayashi, T.; Tsuji, T. *J. Antibiot.* **2001**, *54*, 102–104.
(14) Shibata, K.; Kashiwada, M.; Ueki, M.; Taniguchi, M. *J. Antibiot.* **1993**, *46*, 1095–1100.
(15) Dai, J.-R.; Carté, B. K.; Sidebottom, P. J.; Yew, A. L. S.; Ng, S.-B.; Huang, Y.; Butler, M. S. *J. Nat. Prod.* **2001**, *64*, 125–126.
(16) Mohr, N.; Budzikiewicz, H.; Korth, H.; Pulverer, G. *Liebigs Ann. Chem.* **1981**, 1515–1518.
(17) The NH group at position 3 in the proposed quinazolinone structure is subject to keto−enol tautomerism and could neither be detected in pyridine-d_5 nor in dimethylsulfoxide-d_6 (see Supporting Information).
(18) Noguchi, A.; Kitamura, T.; Onaka, H.; Horinouchi, S.; Ohnishi, Y. *Nat. Chem. Biol.* **2010**, *6*, 641–643.
(19) Itoh, Y.; Haneishi, T.; Arai, M.; Hata, T.; Aiba, K.; Tamura, C. *J. Antibiot.* **1978**, *31*, 838–846.
(20) Niementowski, S. *J. Prakt. Chem.* **1895**, *51*, 564–572.
(21) Le Roes-Hill, M.; Goodwin, C.; Burton, S. *Trends Biotechnol.* **2009**, *27*, 248–258.
(22) Suzuki, H.; Furusho, Y.; Higashi, T.; Ohnishi, Y.; Horinouchi, S. *J. Biol. Chem.* **2006**, *281*, 824–833.