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Chromophenazines from the Terrestrial *Streptomyces* sp. Ank 315†

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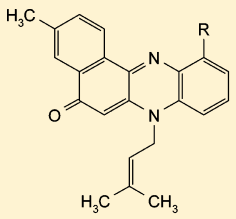
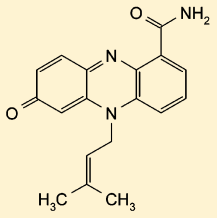
Supporting Information



1a R = H
1b R = COOH



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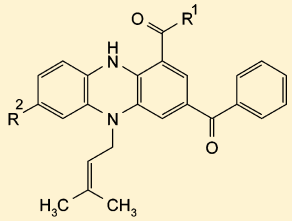

5a R^1 = OH, R^2 = H
5b R^1 = OH, R^2 = C_6H_5CO–
5c R^1 = NH_2, R^2 = C_6H_5CO–

ABSTRACT: The new chromophenazines A–F [9-methyl-5-(3′-methylbut-2′-enyl)-5*H*-benzo[*a*]phenazin-7-one (**1a**), 9-methyl-5-(3′-methylbut-2′-enyl)-7-oxo-5,7-dihydrobenzo[*a*]phenazine-1-carboxylic acid (**1b**), 5-(3′-methylbut-2′-enyl)-7-oxo-5,7-dihydrophenazine-1-carboxamide (**2**), 3-benzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxylic acid (**5a**), 3,7-dibenzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxylic acid (**5b**), and 3,7-dibenzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxamide (**5c**)], together with phenazine-1-carboxylic acid, 1-phenazinecarboxamide, 1-phenazinol, tryptophol, and anthranilic acid, were isolated from *Streptomyces* sp. Ank 315. The structures of the new compounds were established on the basis of spectroscopic data, 1D NOE, 2D NMR, and ESIMS measurements and comparison with literature values.

About 100 phenazine derivatives have been isolated from microorganisms belonging to a wide range of genera, including *Burkholderia, Methanosarcina, Pantoea, Pelagobacter, Pseudomonas, Streptomyces, Vibrio,* and others.[1−6] Simple phenazines have been isolated mostly from *Pseudomonas* species, whereas more complex substituted phenazines are produced by *Streptomyces* spp. (e.g., *S. antibioticus, S. griseolutein, S. luteogriseus,* and *S. prunicolor*).[7] Phenazines display a broad range of activities and act as antioxidant, neuroprotectant, broad-spectrum antimicrobial, antiparasitic, antiviral, antitumor, and antimalarial agents, affecting a wide range of target organisms.[8,9]

In an ongoing investigation of metabolites from microorganisms, extracts of the *Streptomyces* sp. isolate Ank 315 showed pink, orange, and red zones on TLC in daylight, which became colorless on spraying with anisaldehyde/sulfuric acid. A batch culture was grown in 25 and 60 L scales on M_2 medium at 28 °C for eight days. Fractionation of the crude extract on silica gel and Sephadex LH-20 and by preparative TLC afforded six new phenazine derivatives, named chromophenazines A–C (**1a**–**2**) and D–F (**5a**–**5c**). Additionally, phenazine-1-carboxylic acid, 1-phenazinecarboxamide, 1-phenazinol, tryptophol, and anthranilic acid were obtained and identified by means of the AntiBase data system.[10] Details of the isolation are shown in Schemes S1 and S2 (Supporting Information).

RESULTS AND DISCUSSION

Compound **1a** was isolated as an orange powder with long-wavelength UV absorptions at λ_{max} 463 sh, 490, and 519 sh nm. It showed an intense orange fluorescence on TLC under UV at 366 nm and became colorless after spraying with anisaldehyde/H_2SO_4. The molecular mass was determined by ESIMS in both the positive and negative modes, and the molecular formula, $C_{22}H_{20}N_2O$, was obtained by HRESIMS of the $[M + H]^+$ signal (m/z 329.16498). The ^{1}H NMR spectrum of **1a** (Table 1; Figure S2, Supporting Information) displayed seven aromatic protons that could be assigned to two patterns (Figure S1, Supporting Information): The first one showed partially overlapping *ortho*-coupled 1H doublets at δ 7.96 (dd, J = 7.9, 1.5 Hz) and 7.32 (d, J = 7.9 Hz) and triplets at δ 7.36 and 7.56, indicating a 1,2-disubstituted benzene ring (ring A in **1a**). The second pattern consisted of 1H doublets at δ 8.74 (J = 8.2 Hz) and 8.14 (J = 1.7 Hz) and a dd signal at δ 7.54 (J = 8.2, 1.7 Hz),

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Table 1. ^{1}H, ^{13}C, and 2D NMR Data for Chromophenazines A (1a) and B (1b) in $CDCl_3$

	chromophenazine A (**1a**)				chromophenazine B (**1b**)
position	δ_H[a] (J in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)	δ_H[a] (J in Hz)
1	7.96 (dd, 7.9, 1.5)	130.8	3, 4a	2	
2	7.36 (t, 7.9)	123.6	4, 12a	1, 3	8.34 (d, 8.1)
3	7.56 (t, 7.9)	130.9	4a	2, 4	7.71 (t, 8.2)
4	7.32 (d, 7.9)	113.4	2, 3, 12a	3	7.56 (d, 8.4)
4a		131.3			
5a		139.2			
6	6.12 s	99.4	5a, 7, 7a, 11b		6.19 s
7		181.9			
7a		132.7			
8	8.14 (d, 1.7)	125.4	7, 10, 11a, Me-9		8.22 s
9		141.7			
Me-9	2.52 s	21.9	8, 9, 10		2.58 s
10	7.54 (dd, 8.2, 1.7)	131.8	8, 11a, Me-9	11	7.64 (d, 8.3)
11	8.74 (d, 8.2)	125.0	7a, Me-9,11b	10	8.41 (d, 8.2)
11a		129.5			
11b		146.9			
12a		135.0			
1′	4.76 (d, 5.4)	45.7	4a, 5a, 2′	2′, Me-4′, Me-5′	4.80 (d, 5.4)
2′	5.14 m	116.5	Me-4′, Me-5′	1′	5.13 (t, 5.5)
3′		138.2			
Me-4′	1.77 s	25.6	2′, 3′, Me-5′	1′, Me-5′	1.80 s
Me-5′	1.91 s	18.7	2′, 3′, Me-4′	1′, Me-4′	1.93 s

[a] ^{1}H NMR spectra recorded at 300 MHz. [b] ^{13}C NMR spectra recorded at 125 MHz. [c] HMBC and COSY spectra recorded at 600 MHz.

suggesting a 1,3,4-trisubstituted benzene ring (ring D in **1a**); both assignments were confirmed by COSY and HMBC correlations. An additional 1H singlet was found at δ 6.12, and a 3H singlet at δ 2.52 indicated the presence of an *ar*-Me group. The ^{13}C NMR (Figure S3, Supporting Information) and HSQC spectra of **1a** (Table 1) disclosed a total of 22 carbon signals, corresponding to three methyls, one methylene, nine sp^2 methines, and nine quaternary sp^2 carbon atoms. The downfield signal at δ 181.9 indicated the presence of a conjugated ketone. The signals at δ 138.2 (C_q-3′), 116.5 (C-2′; δ_H 5.14, m), 45.7 (C-1′; δ_H 4.76, d, J = 5.4 Hz, CH_2), 25.6 (δ_H 1.77, Me-4′), and 18.7 (δ_H 1.91, Me-5′) pointed to the presence of a prenyl group, which was obviously connected to nitrogen, as the shift of the methylene group indicated.[11,12]

The substituent pattern of ring C followed mainly from HMBC cross-peaks of H-6 with C-5a, -7, -7a, and -11b, respectively. Proton H-8 showed *meta*-coupling with H-10 (which itself had an *ortho* coupling with H-11), gave a long-range COSY coupling with Me-9, and showed HMBC cross-peaks with the C-7 carbonyl, C-9, C-10, and C-11; Me-9 coupled with C-8, C-9, and C-10. This resulted in a 4*H*-naphthalen-1-one subunit, which formed rings C and D in structure **1a** and was further supported by HMBC correlations of Me-9, H-10, and H-11 (Table 1 and Figures S1 and S4, Supporting Information).

HMBC cross-peaks (Figure S4, Supporting Information) from the CH_2-1′ group to C-4a and C-5a, respectively, indicated the position of the *N*-prenylamine subunit. The position of this group was confirmed by 1D NOE experiments, in which irradiation of the methylene-1′ signal (δ 4.80) showed an enhancement of the H-4 (δ 7.32) and H-6 (δ 6.12) signals. In the same way, the shift assignment of the geminal methyl groups was determined. According to the molecular formula, all atoms except one nitrogen atom had been used.

In order to explain the orange color of this compound, a ring closure between A and C forming a phenoxazinone, a 10*H*-acridin-3-one, or a 10*H*-phenazin-2-one must be assumed. The presence of a phenoxazinone (the chromophore of actinomycins) or a 10*H*-acridin-3-one was excluded by the elemental composition; the latter structure was also unlikely, as compounds of this type have never been isolated from Nature. The UV data of **1a**, however, strongly resembled those of endophenazine B (**3**) and suggested a phenazinone.[13] Although all 2D correlations fitted **1a**, further structures may do the same. We searched therefore for alternatives using the 2D NMR evaluation program COCON:[14] Sixteen isomers were obtained, but all except **1a** were metacyclophanes, benzvalenes, calicenes, or other highly strained structures and were excluded on the basis of their ground-state energy (given by COCON). Compound **1a** was thereby identified as 9-methyl-5-(3′-methylbut-2′-enyl)-5*H*-benzo[*a*]phenazin-7-one,[15] for which we suggest the name chromophenazine A.

A second minor component, **1b**, was isolated as an orange powder with an intense orange fluorescence on TLC under UV (366 nm) as well. The UV and NMR data were very similar to those of **1a**, but ring A was 1,2,3-trisubstituted instead of 1,2-disubstituted, as indicated by two clearly separated doublets and a triplet in the aromatic region of the ^{1}H NMR spectrum (Table 1 and Figure S5, Supporting Information). The molecular formula, $C_{23}H_{20}N_2O_3$, derived by HRESIMS of the pseudomolecular ion peak $[M + H]^+$ (m/z 373.15459) differs by CO_2 from **1a**, and the ^{1}H NMR data pointed to a carboxylic group at position 1 or 4 in ring A. ^{13}C or 2D NMR data could not be obtained due to the small amount available, but an NOE signal between CH_2-1′ and both H-4/6 confirmed the prenyl residue again at N-5 and the carboxy group at C-1. The assignment of chromophenazine B as 9-methyl-5-(3′-methylbut-2′-enyl)-7-oxo-5,7-dihydrobenzo[*a*]phenazine-1-carboxylic acid (**1b**) is based on the empirical formula, the UV/vis data, the similarity of the shifts of H-2–H-4 to those of the respective protons in **2** and in endophenazine B[13] (Table S1), and the similarity between **1a**/**1b** for the remaining proton signals.

For the biosynthesis of **1a** and **1b**, a 5,9-diprenylated phenazine precursor related to **3** or to the aglycone of aestivophoenin C,[16] can be postulated, for which the cyclization would give rise to ring D in **1a** and **1b**, respectively.

The violet chromophenazine C (**2**) showed UV/vis absorptions at 224, 281, 361, and 531 nm. The molecular formula, $C_{18}H_{17}N_3O_2$, was derived by HRESIMS of the pseudomolecular ion peak $[M + H]^+$ (m/z 308.13960). The ^{13}C NMR/HSQC data of **2** (Table 2) disclosed 18 carbon signals, corresponding to two methyls, one methylene, seven methines, and eight quaternary carbon atoms (Figure S7, Supporting Information). The downfield signals at δ 183.9 and 166.3 indicated the presence of a conjugated ketone and an

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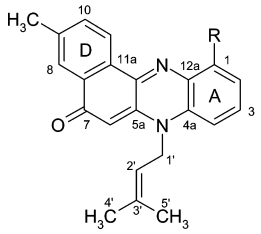
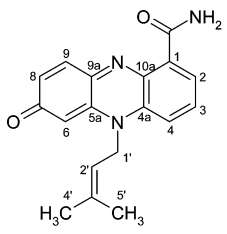


1a: R = H
1b: R = COOH



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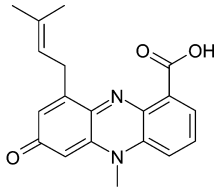

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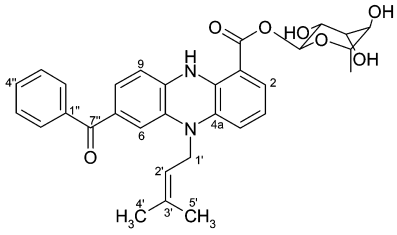

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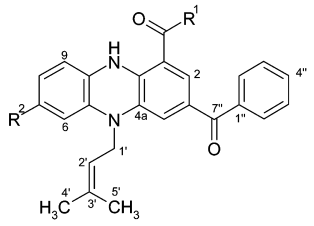
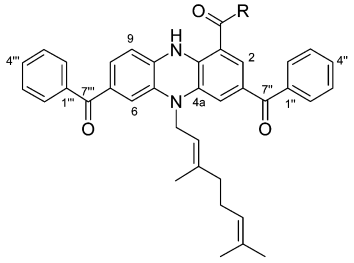


	R^1	R^2
5a	OH	H
5b	OH	C_6H_5CO–
5c	NH_2	C_6H_5CO–



6a: R = OH
6b: R = OMe

amide or acid group. The ^{1}H NMR spectrum of **2** (Figure S6, Supporting Information) displayed signals characteristic of a 1,2,3-trisubstituted benzene ring, with similar shifts to those observed for **1b**. In addition, a doublet at δ 7.58 (1H, d, J = 7.6 Hz), doublets of a doublet at δ 7.12 (1H, J = 7.6, 1.2 Hz), and a narrow doublet at δ 6.16 (1H, d, J = 1.2 Hz) pointed to a 1,2,4-trisubstituted benzene ring. The presence of an *N*-prenyl group was again suggested by signals at δ 5.10 (1H, m), 4.81 (2H, d, J = 4.8 Hz), 1.96 (3H, s), and 1.78 (3H, s) (Table 2). The position of the prenyl group was confirmed as being the same as in **1a** and **1b** by $^3J_{C,H}$ correlations of CH_2-1′ (δ 4.81) with C-4a (δ 131.8) and C-5a (δ 137.1). This was again supported by an NOE enhancement of H-4 (δ 7.58) and H-6 (δ 6.16) after irradiation of CH_2-1′ (δ 4.81) and CH-2′. Following a comparison of the NMR data of **2** with those of **1a**, **1b**, and **3**, it became clear that an annulated benzene ring D was missing in **2**. The amide carbonyl (δ 166.3) showed a clear HMBC correlation with H-2 (δ 8.42), thus further confirming its position at C-1 (Figure S8, Supporting Information). The amide proton at δ 6.35 showed a COSY correlation with the second amide proton at δ 9.86. A similar (but not as strongly separated) geminal coupling of amide protons is also found in phenazine-1-carboxamide (see Figure S9, Supporting Information) and has been investigated in detail, e.g., for picolin- and nicotinamide.[17] Further HMBC correlations are depicted in Figure S1 (Supporting Information) and Table 2. On the basis of these data, chromophenazine C was determined as 5-(3′-methylbut-2′-enyl)-7-oxo-5,7-dihydrophenazine-1-carboxamide (**2**) and confirmed by COCON calculations.[14]

As chromophenazine C (**2**) and endophenazine B (**3**) have the same chromophore, the similarity of their UV/vis absorption spectra (λ_{max} = 531 for **2** and 545 nm sh for **3**, respectively) is to be expected. The color of **2** is in agreement with the UV/vis spectra of synthetic products.[18] In addition, DFT calculations[19] explained why **1a** and **1b** absorb at shorter wavelengths than **2**, as their HOMO/LUMO energy difference is substantially larger. The NMR data of **2** and endophenazine B (**3**) are showing the expected similarity (Table S1, Supporting Information).

The 10*H*-phenazin-2-ones are keto tautomers of 2-hydroxyphenazines. As expected from semiempirical calculations,[19] the phenolic hydroxyphenazine tautomers are more stable than the keto forms, and so it is understandable that among the about 30 natural 2- or 3-hydroxyphenazines, respectively, only one has been claimed to be the keto tautomer: in the violet endophenazine B (**3**),[13] N-5 is alkylated as in **1a** and **2**, so that a rearrangement to the more stable phenol is blocked. A single N-5-monosubstituted 3-phenazinol has been described, but in the dark red 1,8-phenazinediol-10-oxide,[20] the long-wavelength absorption at 540 nm is perhaps

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Table 2. ^{1}H, ^{13}C, and 2D NMR Data for Chromophenazines C (2) in $CDCl_3$ and D (5a) in DMSO-d_6 and of Aestivophoenin A (4) in Acetone-d_6[21]

	chromophenazine C (2)				chromophenazine D (5a)				aestivophoenin A (4)	
no.	δ_H[a] (J in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)	δ_H[d] (J in Hz)	δ_C[b]	HMBC[c] (H→C)	COSY[c] (H↔H)	δ_H	δ_C
1		130.5				116.7				109.7
2	8.42 (d, 7.6)	127.8	4, 4a,10a, $CONH_2$	3, 4	7.51 (d, 1.8)	130.7	4, 10a, COR[1], 7″	4	7.08	122.3
3	7.75 (t, 7.6)	132.1	1,2,4,4a,10a	2, 4		135.5			6.55	121.6
4	7.58 (d, 7.6)	117.5	1,2,10a	2, 3, (1′)	6.45 (s br)	109.0	2, 10a	2	6.35	115.4
4a		131.8				134.7				136.7
5a		137.1				134.0				135.5
6	6.16 (d, 1.2)	100.2	5a,7,9a	(1′), 8	6.16 (d br, 7.7)	110.6	7, 8, 5a, 9a	7, 8	6.70	112.9
7		183.9			6.49 (t, 7.7)	121.7	5a, 9	6		132.4
8	7.12 (dd, 7.6, 1.2)	137.2	6,9a	6, 9	6.43 (t, 7.4)	120.8	6, 9a	6, 9	7.00	127.2
9	7.58 (d, 7.6)	133.6	5a,7	8	6.15 (d, 7.4)	112.3	7, 8, 5a, 9a	8	6.50	112.8
9a		147.5				132.7				139.1
10					12.24 (s br)		1, 4a, 5a,9,9a,10a		9.37	
10a		132.9				143.6				140.5
1′	4.81 (d, 4.8)	46.5	2′,3′,4a,5a	2′, 4, 6, Me-4′, Me-5′	3.95 (d, 4.3)	42.9	4a, 5a, 2′, 3′	2′, Me-4′, Me-5′	4.04	44.6
2′	5.10 (m)	115.5	Me-4′, Me-5′	1′, Me-4′, Me-5′	5.03 (m)	119.0	Me-4′, Me-5′	1′, Me-4′, Me-5′	5.08	119.5
3′		139.3				135.4				137.5
Me-4′	1.78 (s)	25.6	2′, 3′, Me-5′	1′, 2′	1.72 (s)	17.8	2′,3′, Me-5′	1′, 2′	1.69	18.0
Me-5′	1.96 (s)	18.8	2′, 3′, Me-4′	1′, 2′	1.70 (s)	25.3	2′,3′, Me-4′	1′, 2′	1.74	25.8
1″						139.0				139.7
2″,6″					7.55 (m)	128.2	2, 4″, 7″	3″, 5″, 4″	7.68	129.9
3″,5″					7.48 (t, 7.0)	127.9	1″	2″, 6″, 4″	7.51	129.0
4″					7.56 (m)	130.5	2″,6″, 7″	3″, 5″, 2″, 6″	7.59	132.2
7″						193.1				194.5
COR[1]		166.3				169.0				166.8
NH_2	9.86, 6.35 (2s)			NH_2						

[a] ^{1}H NMR spectra recorded at 600 MHz. [b] ^{13}C NMR spectra recorded at 125 MHz. [c] HMBC and COSY spectra recorded at 600 MHz. [d] Recorded at 300 MHz.

better explained by the tautomeric 9,10-dihydroxy-10*H*-phenazin-2-one form.

Chromophenazine D (**5a**) was isolated as a dark red solid with absorption bands at 389 and 491 nm in the visible range. The broad signal at 3446 cm^{-1} in the IR spectrum hinted at the presence of a carboxylic group, which was confirmed by a CO signal at δ 169.0. The molecular formula, $C_{25}H_{22}N_2O_3$, was established by HRESIMS of the pseudomolecular ion peak $[M - H]^-$ (m/z 397.15581). Again, the NMR spectra of **5a** (Figures S10 and S11, Supporting Information) indicated the presence of an *N*-prenyl group as in **1a** and **2**, but here along with an additional benzoyl residue (Table 2). In the HMBC spectrum (Figure S12, Supporting Information), a *meta*-split doublet at δ 7.51 showed an HMBC correlation with the acid carbonyl (δ 169.0) as well as with the benzoyl carbonyl (δ 193.1). The coupling partner of δ 7.51, a broadened singlet at δ 6.45 (H-4), gave cross-peaks with the benzoyl carbonyl alone. Both protons showed an HMBC correlation with the downfield signal at δ 143.6 (C-10a). It follows that the carboxy and benzoyl groups are attached to the same ring at C-1 and C-3, respectively. According to the empirical formula and the COSY and HMBC data, ring C was *ortho*-disubstituted (see Table 2 and Figure S1, Supporting Information). The structure was confirmed by further $^{1}H,^{1}H$ COSY and HMBC correlations. As a result, chromophenazine D (**5a**) was elucidated as 3-benzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxylic acid. A search in AntiBase[10] for related compounds resulted in suggested matches with aestivophoenins A (**4**)[21] and B and benthophoenin.[22] The NMR data of **4** were, however, clearly different (Table 2). Chromophenazine D (**5a**) is therefore an isomer of the so far undescribed aglycone of aestivophoenin A (**4**).

The molecular formula of the pink chromophenazine E (**5b**) was determined to be $C_{32}H_{26}N_2O_4$ via HRESIMS of the pseudomolecular ion peak $[M - H]^-$ (m/z 501.18201). The ^{1}H NMR spectrum of **5b** (Figure S13, Supporting Information) showed signals indicative of a 1,2,4-trisubstituted benzene ring, along with *meta*-coupled proton signals characteristic of a tetrasubstituted benzene ring (Table 3). In addition, signals for two benzoyl groups at δ 7.61−7.55 (10H, signals overlapping; two CO at δ 193.4, 193.0) and a carboxy group (δ 168.2) were observed. An *N*-prenyl residue was again found, and a singlet at δ 13.1 indicated NH-10 of a further dihydrophenazine. A substructure search[10] suggested a close similarity to benthophoenin,[12] which had, however, a C_{10}-prenyl residue at N-5 instead of a C_5-prenyl group.

Irradiation into the allyl methylene group C-1′ caused an enhancement of the signals of H-4 and H-6, indicating that N-5 and not N-10 must be prenylated as in the previous chromophenazines. The attachment of the carboxy group at

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Table 3. ^{1}H and ^{13}C NMR Data of Chromophenazines E (5b) and F (5c) in DMSO-d_6 in Comparison with Benthophoenin (6a) in CD_3OD and Benthophoenin Methyl Ester (6b) in $CDCl_3$

	chromophenazine E (**5b**)		chromophenazine F (**5c**)		benthophoenin (**6a**)		benthophoenin methyl ester (**6b**)	
position	δ_H[a] (J in Hz)	δ_C[b]	δ_H[c] (J in Hz)	δ_C[b]	δ_H[d] (J in Hz)	δ_C[b]	δ_H[d] (J in Hz)	δ_C[b]
1		118.2		109.4		124.9		107.5
2	7.51 (d, 1.2)	130.9	7.10 (d, 1.4)	123.9	7.73	131.8	7.53	127.5
3		130.0		129.4		136.8		129.5
4	6.50 (d, 1.8)	110.6	6.52 (br s)	113.0	n.p.[e]	112.7	6.67	113.2
4a		134.6		136.4		132.8		136.1
5a		134.1		134.7		132.8		134.8
6	6.53 (d, 1.1)	109.6	6.72 (d 1.2)	112.0	6.69	113.0	6.76	112.4
7		129.6		132.2		135.9		132.7
8	6.87 (dd, 7.9,1.8)	126.7	6.93 (dd, 7.9,1.4)	127.0	6.99	128.3	6.98	126.6
9	6.23 (d, 7.9)	111.2	6.20 (d, 7.9)	112.3	6.32	112.9	6.25	112.4
9a		138.6		136.8		139.9		136.4
10	13.12 (s)		10.3 (s)				9.80	
10a		141.5		142.8		143.8		143.8
1′	3.94 (d, 5.4)	43.0	3.91 (d, 6.3)	44.2	n.p.[e]	44.9		44.2
2′	5.03 (td, 5.9, 1.4)	117.2	4.99 (m)	117.4	5.06	119.5	5.02	117.1
3′		136.9		137.5		141.7		141.4
Me-4′	1.59 (s)	17.6	1.50 (s)	17.8	2.08	16.5	2.08	16.3
Me-5′	1.71 (s)	25.4	1.68 (s)	25.8	1.68	40.5	1.62	39.5
6′					2.08	27.2	2.01	26.4
7′					5.06	124.9	5.02	123.7
8′						132.5		131.7
9′					1.61	17.8	1.61	17.7
10′					1.59	25.8	1.56	25.6
1″		138.7[f]		137.9[f]		139.9		138.4
2″, 6″	7.60[f]	128.5[f]	7.67 (d, 7.8)[f]	129.3[f]	7.66	130.3	7.69	129.4
3″, 5″	7.50[f]	128.1[f]	7.45 (t, 7.8)[f]	128.0[f]	7.50	129.3	7.43	128.1
4″	7.60[f]	131.1[f]	7.51 (tt, 7.5,1.2)[f]	131.5[f]	7.59	132.6	7.53	131.7
7″		193.4[f]		194.1[f]		197.1		194.8
1‴		138.3[f]		138.3[f]		139.9		138.1
2‴,6‴	7.60[f]	128.4[f]	7.67 (d, 7.8)[f]	129.2[f]	7.66	130.3	7.69	129.4
3‴, 5‴	7.50[f]	128.0[f]	7.42 (t, 7.8)[f]	128.2[f]	7.50	129.3	7.45	128.1
4‴	7.60[f]	130.9[f]	7.54 (tt, 7.5,1.3)[f]	131.8[f]	7.59	132.6	7.55	131.7
7‴		193.0		194.7		197.1		194.2
COR^1-1		168.2	6.0, 5.4 (s, br)[g]	170.2		173.8		168.2
CO_2Me-1							3.81	52.1

[a]Recorded at 300 MHz. [b]Recorded at 125 MHz. [c]Recorded at 600 MHz. [d]Recorded at 500 MHz. [e]n.p. = not published. [f]The values of the benzoyl ring shifts could not be distinguished and are tentatively assigned according to calculations using ACD. [g]Tentatively assigned to $CONH_2$

C-1 and a benzoyl group at C-3 was confirmed by HMBC spectra (Figure S16, Supporting Information), in which H-2 (δ 7.51) showed ^{3}J correlations with the acid carbonyl (δ 168.2) and the benzoyl carbonyl (δ 193.4). The position of the second benzoyl residue at C-7 was derived from ^{3}J correlations of H-6 (δ 6.53) and H-8 (δ 6.87) with the carbonyl at δ 193.0. Thus, the structure of chromophenazine E was established as 3,7-dibenzoyl-5-(3-methylbut-2-enyl)-5,10-dihydrophenazine-1-carboxylic acid (**5b**).

Chromophenazine F (**5c**) was isolated as a red powder, with a UV/vis band at λ_{max} = 511 nm at long wavelength. The molecular formula, $C_{32}H_{27}N_3O_3$, was determined by HRESIMS of the pseudomolecular ion peak $[M - H]^-$ (m/z 500.19823). The ^{1}H NMR spectrum of **5c** (Table 3 and Figure S17, Supporting Information) was nearly identical with that of **5b**, and the 2D NMR data led to the same carbon skeleton (Figures S1, S18, and S20, Supporting Information). With respect to the empirical formula, however, the signal at δ 170.2 was assigned to an amide group instead of an acid, so that chromophenazine F (**5c**) is 3,7-dibenzoyl-5-(3-methylbut-2-enyl)-5,10-dihydrophenazine-1-carboxamide; the amide proton signals were not visible in this case.

Chromophenazines E (**5b**) and F (**5c**) are closely related to benthophoenin (**6a**) isolated from *Streptomyces prunicolor* and to the methyl ester **6b** thereof.[11] Their ^{1}H and ^{13}C NMR data resemble those of the benthophoenins, as expected (Table 3). Especially between **5c** and **6b**, there is an obvious close similarity. Surprisingly, in the positive-ion mode electrospray mass spectra of chromophenazines D (**5a**), E (**5b**), and F (**5c**), strong signals were observed corresponding to the odd-electron $M^{+\bullet}$ ions. The identity of these species was confirmed by detailed HRESIMS measurements. Additional $[M + H]^+$ ions were detected with low intensity for chromophenazines E (**5b**) and F (**5c**), but not for chromophenazine D (**5a**). The formation of the $[M]^{+\bullet}$ species was not observed for chromophenazines A (**1a**), B (**1b**), and C (**2**).

Bioassays of the pure compounds using an agar diffusion assay (40 μg/disk) were carried out against the bacteria *Bacillus*

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subtilis, *Escherichia coli*, and *Staphylococcus aureus*, the fungus *Mucor miehei* (Tü284), and the yeast *Candida albicans*. Only chromophenazine D (**5a**) showed moderate activity against *B. subtilis*, *E. coli*, and *M. miehei*, with inhibition zones of 10 mm each. This resembles the activity of endophenazines A and C, which also showed weak antimicrobial activity against several Gram-positive bacteria and some fungi.[13] Chromophenazines A (**1a**), B (**1b**), C (**2**), and F (**5c**) did not show any activity in the tests used, as was seen for endophenazine B.[13] The potent activity of the crude extract of *Streptomyces* sp. Ank 315 against the tested strains was mainly due to phenazinecarboxylic acid and phenazinol.

EXPERIMENTAL SECTION

General Experimental Procedures. UV/vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/vis spectrometer. IR spectra were recorded on a Perkin-Elmer (model 1600) FTIR spectrometer. NMR spectra were recorded on Varian Unity 300 (^{1}H, 300.145 MHz) and Varian Inova 600 (599.740 MHz) NMR spectrometers. Electrospray-ionization mass spectrometry (ESIMS) and high-resolution mass spectra (HRESIMS) were recorded on a micrOTOF time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany), as well as on an Apex IV 7 T Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA). TLC was carried out on precoated silica gel sheets of Polygram SIL G/UV$_{254}$ (Macherey-Nagel and Co., Düren, Germany). Size-exclusion chromatography was performed on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd.; purchased from Sigma-Aldrich Chemie, Steinheim, Germany). Amberlite XAD-16 resin was obtained from Rohm and Haas, Frankfurt, Germany.

Isolation and Taxonomy. The *Streptomyces* sp. strain Ank 315 was derived from a soil sample and isolated on YMG agar at room temperature (YMG agar: 2 g/L yeast extract, 5 g/L malt extract, 5 g/L glucose, 15 g/L agar, 30 mg/L cycloheximide). Its almost complete 16S rRNA gene sequence (GenBank accession no. HM367878) shows high similarities to *Streptomyces galbus* strain NBRC 12864 (GenBank accession no. AB184201). The strain is deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany.

Fermentation and Isolation. *Streptomyces* sp. Ank 315 was cultivated on a 25 and 60 L scale using 1 L Erlenmeyer flasks containing 250 mL of M$_2$ medium (malt extract 10 g, yeast extract 4 g, glucose 4 g per liter tap water; adjusted to pH 7.8 with 2 N NaOH) at 28 °C for eight days on a linear shaker (250 rpm). The culture broth was mixed with Celite and filtered with a filter press. The filtrate was passed through an Amberlite XAD-16 column (120 × 5.5 cm), and the resin was washed with distilled H$_2$O and eluted with MeOH. The methanol phase was concentrated, and the aqueous residue was extracted with EtOAc. The mycelium was extracted sequentially with EtOAc and then acetone. The extracts showed similar compositions on TLC and were combined. Compounds **1a** and **1b** were obtained from the 25 L cultivation, whereas compounds **2** and **5a**–**5c** were isolated from the 60 L cultivation. Additionally, the following known compounds were isolated: phenazine-1-carboxylic acid (40 mg), 1-phenazinol (25 mg), 1-phenazinecarboxamide (15 mg), anthranilic acid (12 mg), and tryptophol (10 mg). Further details of the isolation are shown in Schemes S1 and S2 (Supporting Information).

Chromophenazine A (9-methyl-5-(3′-methylbut-2′-enyl)-5*H*-benzo[*a*]phenazin-7-one, 1a): orange powder, 1.5 mg (from 25 L), 5 mg (from 60 L), R_f 0.36 (5% MeOH/CH_2Cl_2); UV/vis (MeOH) λ_{max} (log ε) 229 (4.91), 256 (4.20), 271 (4.02), 278 (4.04), 308 (4.16), 372 (3.76), 463 sh (3.91), 490 (4.02), 519 sh (3.87) nm; IR (KBr) ν_{max} 3429, 2923, 2854, 1583, 1544, 1460, 1377, 1321, 1233, 1161, 1055 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$), and 2D data, see Table 1 and Figures S2–S4, Supporting Information; (+)-ESIMS *m*/*z* 329 ([M + H]$^+$, 100%); (+)-HRESIMS *m*/*z* 329.16498 [M + H]$^+$ (calcd for $C_{22}H_{21}N_2O$, 329.16484).

Chromophenazine B (3-methyl-7-(3′-methylbut-2′-enyl)-5-oxo-5,7-dihydrobenzo[*a*]phenazine-1-carboxylic acid, 1b): orange powder, 0.5 mg from 25 L, R_f 0.28 (5% MeOH/CH_2Cl_2); UV/vis (MeOH) λ_{max} (log ε) 247 sh (4.22), 277 sh (4.04), 301 (3.98), 309 (3.98), 363 (3.62), 491 (3.39), 521 sh (3.28) nm; ^{1}H NMR (600 MHz, $CDCl_3$) data, see Table 1 and Figure S5, Supporting Information; (+)-ESIMS *m*/*z* 767 ([2 M + Na]$^+$, 25%), 395 ([M + Na]$^+$, 100%); (−)-ESIMS *m*/*z* 371 ([M − H]$^-$; (+)-HRESIMS *m*/*z* 373.15459 [M + H]$^+$ (calcd for $C_{23}H_{21}N_2O_3$, 373.15467).

Chromophenazine C (5-(3′-methylbut-2′-enyl)-7-oxo-5,7-dihydrophenazine-1-carboxamide, 2): violet powder, 2.3 mg from 60 L, R_f 0.20 (7% MeOH/CH_2Cl_2); UV/vis (MeOH) λ_{max} (log ε) 224 (3.48), 281 (3.34), 361 (2.84), 531 (2.80) nm; IR (KBr) ν_{max} 3446, 2928, 1736, 1718, 1654, 1541, 1459, 1384, 589 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6), ^{13}C NMR (125 MHz, DMSO-d_6), and 2D data, see Table 2 and Figures S6–S8, Supporting Information; (+)-ESIMS *m*/*z* 637 ([2M + Na]$^+$, 95%), 330 ([M + Na]$^+$, 100%); (−)-ESIMS *m*/*z* 306 ([M − H]$^-$); (+)-HRESIMS *m*/*z* 308.13960 [M + H]$^+$ (calcd for $C_{18}H_{18}N_3O_2$, 308.13935).

Chromophenazine D (3-benzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxylic acid, 5a): dark red solid, 3.8 mg from 60 L, R_f 0.13 (5% MeOH/CH_2Cl_2); UV/vis (MeOH) λ_{max} (log ε) 247 (4.20), 301 (4.02), 389 (3.50), 491 (3.62) nm; IR (KBr) ν_{max} 3447, 2925, 2373, 2080, 1836, 1560, 1495, 1292, 587 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6), ^{13}C NMR (125 MHz, DMSO-d_6), and 2D data, see Table 2 and Figure S10–S12, Supporting Information; (−)-ESIMS *m*/*z* 817 ([2M + Na − 2H]$^-$, 50%), 397 ([M − H]$^-$, 100%); (−)-HRESIMS *m*/*z* 397.15581 [M − H]$^-$ (calcd for $C_{25}H_{21}N_2O_3$, 397.15577); (+)-HRESIMS *m*/*z* 398.16241 [M]$^{\bullet+}$ (calcd for $C_{25}H_{22}N_2O_3$, 398.16249).

Chromophenazine E (3,7-dibenzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxylic acid, 5b): pink solid, 4 mg from 60 L, R_f 0.30 (5% MeOH/CH_2Cl_2); UV/vis (MeOH) λ_{max} (log ε) 249 (4.09), 310 (4.16), 387 (3.95), 522 (3.72) nm; IR (KBr) ν_{max} 3448, 2927, 1653, 1636, 1542, 1497, 1424, 1274, 1118, 890, 722 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6), ^{13}C NMR (125 MHz, DMSO-d_6), and 2D data, see Table 3 and Figures S13–S16, Supporting Information; (−)-ESIMS *m*/*z* 1025 ([2M − 2H + Na]$^-$, 12%), 501 ([M − H]$^-$, 40%), 432 ([M − C_5H_{10}]$^-$, 100%); (−)-HRESIMS *m*/*z* 501.18201 [M − H]$^-$ (calcd for $C_{32}H_{25}N_2O_4$, 501.18198); (+)-ESIMS *m*/*z* 503 ([M + H]$^+$, 33%), 502 ([M]$^{\bullet+}$, 100%).

Chromophenazine F (3,7-dibenzoyl-5-(3′-methylbut-2′-enyl)-5,10-dihydrophenazine-1-carboxamide, 5c): red powder, 3.6 mg from 60 L, R_f 0.26 (5% MeOH/CH_2Cl_2); UV/vis (MeOH) λ_{max} (log ε) 251 (3.84), 304 (3.76), 394 (3.15), 511 (3.24) nm; IR (KBr) ν_{max} 3429, 2923, 2853, 1641, 1544, 1496, 1446, 1384, 1275, 1128, 715 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6), ^{13}C NMR (125 MHz, DMSO-d_6), and data, see Table 3 and Figures S18–S20, Supporting Information; (−)-ESIMS *m*/*z* 500 ([M − H]$^-$); (−)-HRESIMS *m*/*z* 500.19823 [M − H]$^-$ (calcd for $C_{32}H_{26}N_3O_3$, 500.19797); (+)-ESIMS *m*/*z* 1025 ([2M + Na]$^+$, 100%), 524 ([M + Na]$^+$, 75%); 502 ([M + H]$^+$, 30%), 501 ([M]$^{\bullet+}$, 100%); (+)-HRESIMS *m*/*z* 524.19472 [M + Na]$^+$ (calcd for $C_{32}H_{27}N_3O_3Na$ 524.19446).

Agar Diffusion Bioassay. This procedure was carried out as described previously.[23]

ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, and some 2D NMR spectra of chromophenazines A (**1a**), C (**2**), and D–F (**5a**–**5c**); ^{1}H NMR spectra of chromophenazine B (**1b**) and phenazine-1-carboxamide. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

[†]Dedicated to Prof. Dr. Dr. Gerhard Bringmann on his 60th birthday.

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