New Benz[a]anthraquinone Secondary Metabolites from *Streptomyces phaeochromogenes*

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Four new benz[a]anthraquinone metabolites related to PD 116198, a known benz[a]anthraquinone antibiotic, were isolated from *Streptomyces phaeochromogenes* WP 3688 and characterized. Two showed strong structural similarities with PD 116198, while two others were less similar and possessed novel oxidized skeletons. The relative stereochemistry of these metabolites was obtained by analysis of difference NOE and NOESY spectra.

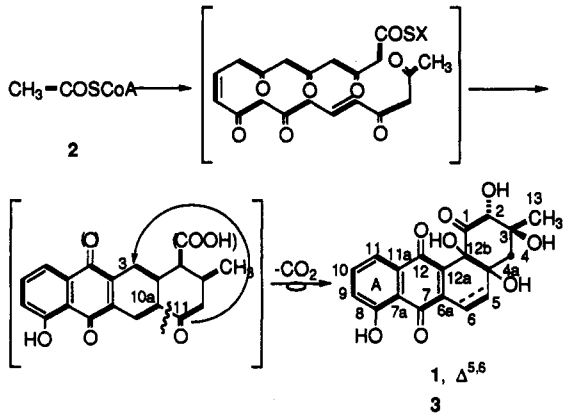
The benz[a]anthraquinone (angucycline) antibiotics are a rapidly growing group of bioactive natural products.[1] We have previously reported[2] results from initial biosynthetic experiments on PD 116198,[3] **1**, a yellow benz[a]-anthraquinone antibiotic produced by *S. phaeochromogenes* WP 3688. The D ring labeling pattern indicated that **1** is not derived from acetylCoA, **2**, by simple condensation and folding of a decaketide intermediate directly to the angular benz[a]anthraquinone skeleton.[4–6] Instead, it appears to be formed via a novel, unexpected rearrangement of a linear tetracyclic intermediate (Scheme 1).

Oxidative elaboration of the D-ring, most likely at late stage(s) of the pathway, appeared necessary to account for at least some of the four hydroxyl groups. We have investigated other colored metabolites produced by this organism in order to learn more about this unusual pathway. Central to this effort has been the investigation of the effectiveness in *S. phaeochromogenes* fermentations of inhibitors of mono- and dioxygenases involved in biological hydroxylations. A number of new metabolites have been isolated, two with quite unusual structures. We now report the structures of four of these compounds.

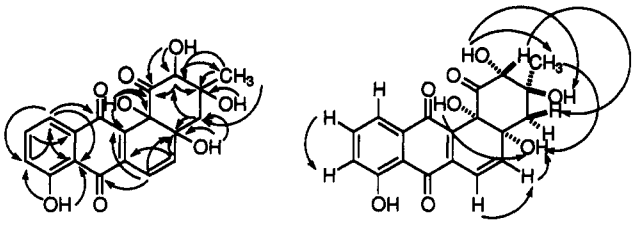
Results and Discussion

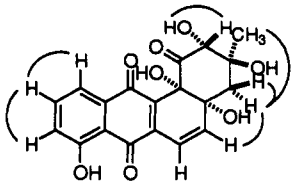
The structure of **1** had been previously reported exclusive of stereochemistry.[3] To this extent, it appeared identical to yoronomycin[7] and sakyomicin B.[8] Considering the unusual biosynthetic findings, confirmation of the structure was deemed prudent. All efforts failed to provide a crystal of **1**, its dihydro derivative **3**, or a heavy-atom or chiral ester of either that was suitable for X-ray diffraction analysis. However, a series of 1D and 2D NMR experiments confirmed the structure and provided the relative stereochemistry. The critical assignments were obtained from an heteronuclear multiple bond correlation (HMBC)[9]

Scheme 1



experiment, which yielded the ^{1}H–^{13}C correlations shown in **1a**, and from NOESY and difference NOE experiments (1.6–15.0% enhancements), which gave the NOE's shown in **1b**. Additional NOE's from the latter are shown in **1c**. Thus, **1** ($[\alpha]_D$ −41.6° in dioxane) appears to be the enantiomer of sakyomicin B ($[\alpha]_D$ +31.6° in dioxane). Yoronomycin ($[\alpha]_D$ +73.7° in dioxane) is apparently a diasteriomer. An A-ring C-nucleoside/D-ring diastereomer (dioxamycin) has also been described.[10]





Minor amounts of numerous other colored metabolites were observed in extracts of *S. phaeochromogenes* grown

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(9) HMQC stands for heteronuclear multiple quantum correlation, and HMBC stands for heteronuclear multiple bond correlation.

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Table 1. ^{1}H NMR Spectral Data of *Streptomyces phaeochromogenes* Metabolites

	^{1}H δ mult (J, Hz)					
hydrogen	1[a]	4[a]	4[b]	5[a]	6[a]	7[a]
1-OH						5.95 s, 4.51 d (6.5)
2a	4.20 d (6.2)	c	2.46 d (12.0)	3.75 d (3.7)	5.75 s	3.78 d (6.5)
2b		c	3.01 d (12.0)			
2-OH	4.60 d (6.2)			5.00 d (3.7)		
3-OH	4.06 s	3.60 s		4.40 s		
4a	1.80 dd (14.8, 1.9)	c	1.99 (m)[c]	1.95 brs	2.95 s	2.91 d (14.5)
4b	2.05 d (14.8)	c	2.12 d (19.0)	1.95 brs		2.35 d (14.5)
4a-OH	4.45 d (2.2)	3.75 s			4.60 s	
5a	6.35 (9.8)	c	1.99 (m)[c]	6.05 d (13.3)	2.80 ddd (16.8, 16.8, 8.3)	6.18 d (9.8)
5b		c	2.03 (m)[c]		2.00 ddd (16.8, 16.3, 4.3)	
6a	6.80 (9.8)	c	2.67 ddd (18.0, 8.0, 7.0)	6.20 d (13.3)	1.70 ddd (16.8, 16.3, 4.3)	6.44 d (9.8)
6b		2.90 ddd (9.2, 5.4, 5.4)	2.91 ddd (18.4, 6.0, 6.0)		2.40 ddd (16.8, 16.3, 4.3)	
6a-OH				4.70 s		
8-OH	11.80 s	11.85 s	11.85 s	11.00 s	11.00 s	11.00 s
9	7.30 d (7.9)	7.26 dd (8.2, 1.0)	7.27 dd (9.9, 2.5)	7.30 dd (8.0, 1.0)	7.25 d (8)	7.35 dd (11.0, 1.8)
10	7.66 dd (7.9, 7.9)	7.63 dd (8.1, 7.8)	c	7.70 dd (7.5, 8.0)	7.65 dd (7.5, 8.0)	7.69 dd (10.8, 10.0)
11	7.54 d (7.9)	7.53 dd (7.8, 1.0)	c	7.55 dd (7.5, 1.0)	7.50 d (7.5)	7.63 dd (10.1, 1.5)
12a-OH				5.90 s	5.20 s	
12b-OH	5.30 s	5.40 s	5.40 s			
12b					3.20 s	
13	1.20 s	1.30 s	1.30 s	1.05 s	1.50 s	1.48 s

[a] In dioxane-d_8. [b] In $CDCl_3$. [c] Overlaps with other resonances.

Table 2. ^{13}C NMR Spectral Data *Streptomyces phaeochromogenes* Metabolites

	δ ^{13}C					
carbon	1[a]	4[a]	4[b]	5[a]	6[a]	7[a]
1	206.3 C	205.1 C	205.7 C	207.5 C	204.0 C	91.0 C
2	82.8 CH	52.6 CH_2	50.9 CH_2	76.5 CH	127.1 CH	78.8 CH
3	76.1 C	73.0 C	72.6 C	77.3 C	156.5 C	74.5 C
4	44.0 CH_2	45.5 CH_2	45.1 CH_2	43.7 CH_2	65.0 CH	40.6 CH_2
4a	76.9 C	74.2 C	73.6 C	73.3 C	92.9 C	89.0 C
5	147.3 CH	31.2 CH_2	30.2 CH_2	139.4 CH	33.0 CH_2	133.1 CH
6	117.1 CH	30.0 CH_2	29.9 CH_2	121.3 CH	27.6 CH_2	128.7 CH
6a	138.5 C	143.4 C	141.6 C	75.7 C	56.9 C	93.0 C
7	187.0 C	190.7 C	189.2 C	197.7 C	197.9 C	196.7 C
7a	115.9 C	116.1 C	114.7 C	116.9 C	118.5 C	117.3 C
8	161.9 C	161.7 C	161.2 C	162.3 C	162.7 C	163.2 C
9	124.8 CH	124.5 CH	124.6 CH	125.0 CH	124.0 CH	125.6 CH
10	137.1 C	136.9 CH	136.6 CH	136.9 CH	138.3 CH	137.0 CH
11	119.3 C	119.4 CH	119.6 CH	119.5 CH	118.8 CH	119.3 CH
11a	133.0 C	132.9 C	131.6 C	134.9 C	136.5 C	134.7 C
12	183.4 C	184.1 C	184.2 C	192.9 C	193.6 C	185.4 C
12a	139.1 C	147.5 C	147.7 C	80.4 C	88.0 C	111.1 C
12b	77.5 C	77.2 C	77.0 C	80.6 C	61.6 CH	165.8 C
13	22.4 CH_3	22.8 CH_3	21.8 CH_3	22.2 CH_3	23.5 CH_3	16.3 CH_3

[a] In dioxane-d_8. [b] In $CDCl_3$.

as previously reported.[3] In order to improve the likelihood of finding intermediates in the biosynthesis of 1, we investigated the *in vivo* use of mono- and dioxygenase inhibitors in order to block at least some of the late-stage hydroxylations. Compounds tested included *p*-chloromercuribenzoic acid, diethyl dithiocarbamate, 2,2-dipyridyl, *N*-ethylmaleimide, 8-hydroxyquinoline, *o*-phenanthroline, ancymidol, and metyrapone. Only metyrapone (2-methyl-1,2-dipyrid-3-yl-1-propanone), a cytochrome P_{450} inhibitor, had any substantial effect. Specific inhibitors of cytochrome P_{450} inhibitors have been successfully used in this manner to interfere with oxidations in a number of pathways of secondary metabolism.[11-16] In the present case, HPLC analysis indicated metyrapone decreased accumulation of 1 approximately 10-fold and increased accumulation of another yellow compound, 4, approximately 3-fold. Although only small amounts of 4 were isolated (see Experimental Section), the enhancement was clearly reproducible by analytical HPLC. Using this protocol, we initially isolated usable quantities of two yellow compounds, 4 and 5, from ethyl acetate extracts of 2 L of fermentation broth. These compounds were subsequently identified in a standard fermentation without metyrapone. Scale-up to 15 L allowed isolation of sufficient amounts of both compounds, as well as of three additional, nearly colorless compounds that were strongly fluorescent blue when irradiated with long-wavelength UV light, for structural elucidation. Each compound was purified by silica gel flash chromatography.

High-resolution (HR) negative ion FAB mass spectral analysis of the yellow compound that accumulated in the presence of metyrapone showed a molecular ion at *m/z* 358.1053, which corresponded to the formula $C_{19}H_{18}O_7$. In agreement with the molecular formula, the ^{13}C NMR

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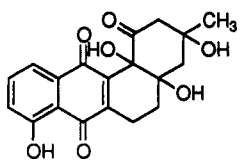
spectrum of 4 contained 19 resonances. Some of the chemical shifts suggested the typical hydroxynaphthoquinone (A/B rings) of an angucycline, as well as a similarity to the C and D rings of 1 (Table 2). A DEPT experiment established the multiplicities of the carbon resonances, while a heteronuclear multiple quantum correlation (HMQC)[9] experiment permitted assignment of the attached protons. Characteristic ABX spin resonances in the ^{1}H NMR spectra of 4 in dioxane-d_8 (Table 1) included doublets at δ 7.26 and 7.53 and a doublet of doublets at δ 7.63, attributable to the A ring protons. An HMBC spectrum of 4 in dioxane-d_8 gave proton–carbon long-range couplings which confirmed the AB-ring partial structure (**4a**). However, the cross peaks to the aliphatic



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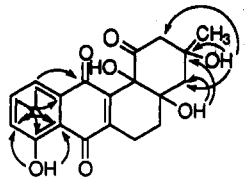
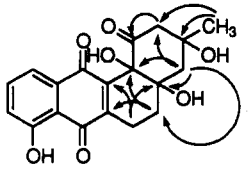
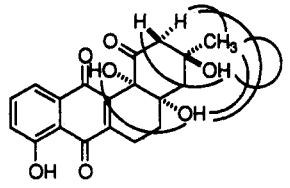


4a



4b



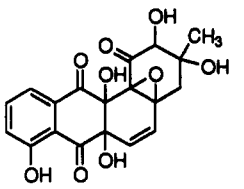
4c

hydrogens were ambiguous due to severe overlapping of the proton resonances from δ 0.8 to 3.0. The HMBC spectrum in $CDCl_3$ yielded the cross peaks indicated in **4b**. Critical crosspeaks from H-6 to C-6a and -12a anchored the CD-rings to the AB-rings. The relative configuration of the C and D rings 4 was determined by difference NOE (1.9–7.3% enhancements) in dioxane-d_8. The observed NOE's for the critical positions are shown in **4c**.

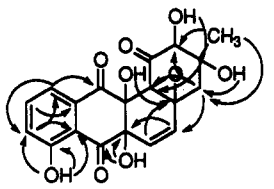
The HR electron impact (EI) mass spectrum of **5** (molecular ion at m/z 388.0793) furnished the molecular formula $C_{19}H_{16}O_9$. The ^{1}H and ^{13}C NMR of **5** also showed remarkable similarities to those of 1 (Tables 1 and 2). ^{1}H and COSY NMR spectra revealed three coupled spin systems, as well as a number of isolated singlets. Five exchangeable resonances were due to four hydroxyl hydrogens and one phenol, and one of the former (δ 5.00) was a doublet coupled to a methine at δ 3.75. The other two coupled systems comprised a typical A-ring pattern (cf. 1 and 3) and a *cis*-disubstituted olefin (J = 13.3 Hz). Long-range couplings could be observed to all four hydroxyl resonances when spectra were obtained in dioxane-d_8.

Three ketone carbonyls were observed in the ^{13}C NMR spectrum of **5**, and their positions were established from the HMBC spectrum. One carbonyl carbon (δ 192.9) correlated with H-11 of the A-ring. Another (δ 197.7) correlated with a tertiary hydroxyl (δ 4.7) that also correlated with its attached carbon (δ 75.7), which in turn correlated with one of the vinyl hydrogens (δ 6.05). The third carbonyl (C-1) correlated directly with the tertiary alcohol at δ 5.9, which in turn could be traced through a series of ^{1}H–^{13}C correlations back to this same carbonyl and to the vinyl system. The chemical shift for C-1, δ 207.5, is characteristic of this position in benz[*a*]anthraquinones. The remaining oxygen was assigned to an epoxide bridging C-4a (δ 73.3) and C-12b (δ 80.6), each of which showed long-range correlations to D-ring hydrogens. These connectivities, shown in **5a**, established the structure.

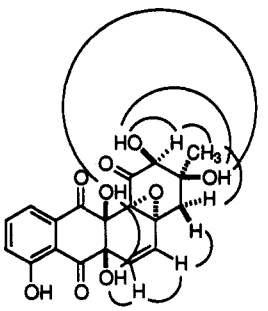
The relative stereochemistry of **5** was established by difference NOE (1.5-8.5% enhancements) and NOESY experiments in dioxane-d_8. As illustrated in **5b**, the hydroxyl at C-3 (axial) gave NOE correlation peaks with the hydroxyl at C-2 (ax), the hydroxyl at C-12a, and the hydrogen at C-5, while the hydroxyl at C-12a also gave an NOE with the hydroxyl at C-6a. Interestingly, the four hydroxyl groups of **5** are proximal to each other in three-dimensional space.



5



5a



5b

The HR EI mass spectrum of **6** (m/z = 372.0844) revealed the formula $C_{19}H_{16}O_8$, and the ^{13}C NMR spectrum showed 19 resonances. Together, these data revealed 12 unsaturations. The presence of three carbonyls and eight additional sp^2 carbons indicated a five ring system. Four partial structures could be discerned. Fragment A (A/B rings) was deduced by an HMBC experiment which revealed the typical $^3J_{CH}$, including from H-11 to C-12 (data not shown). A long-range ^{1}H–^{1}H COSY spectrum also revealed two other spin systems: fragments B and C. The remaining five carbons—three quaternary sp^3 carbons (δ 92.8, 88.0 and 56.8), one sp^3 methine (δ 61.6), and one carbonyl (δ 204)—comprised fragment D. One phenol and two hydroxyl groups were observed in the ^{1}H NMR spectrum, and the data suggested the latter were due to one secondary alcohol and one tertiary alcohol (fragments B and D, respectively). The chemical shift of the quaternary sp^3 carbon at δ 92.9 indicated that it must bear two oxygens; these last oxygens must be shared with the carbons at δ 61.6 and 56.9.

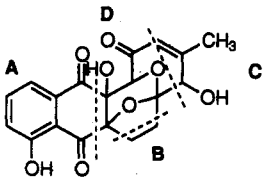
The connectivity of fragments A–D was solved from the HMBC spectrum, which—in addition to typical three-bond correlations—demonstrated a number of four-bond proton–carbon couplings over an oxygen bridge (**6a**): coupling between H-4 (δ 2.95) to C-12b (δ 61.6), between H-12b (δ 3.20) and C-4 (δ 61.6), between H-5 (δ 2.80) and C-12b (δ 61.6), and between H-12b and C-7 (δ 197.5) (not shown). A three-bond correlation between H-12b and the C-12 (δ 193.6) carbonyl was also observed. Additional ^{1}H–^{13}C couplings are shown in **6a**, and the key correlations that linked the four partial structures are given in Table 3. Although observations of four-bond connectivities have been considered unusual, a number of examples have

Table 3. HMBC Connections for Part Structures of 6

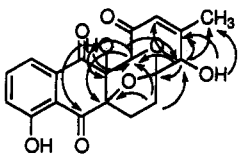
structural fragments	connected by
A and D	H_{12b}–C_{12} ($^3J_{CH}$); H_{12b}–C_7 ($^4J_{CH}$); H_{12aOH}–C_7 ($^4J_{CH}$)
B and D	H_5–C_{6a} ($^3J_{CH}$); H_5–C_{12b} ($^4J_{CH}$)
B and C	H_5–C_4 ($^3J_{CH}$)
C and D	H_4–C_{4a} ($^4J_{CH}$); H_4–C_{12b} ($^4J_{CH}$); H_{12b}–C_4 ($^4J_{CH}$)

recently been reported.[17,18] In addition, at least one example of a 5-bond C–H correlation has also been reported.[19]

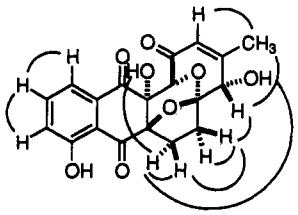
The remaining carbonyl (δ 204) must be at C-1, again this chemical shift is typical of this position (Table 2). Structure **6** was confirmed by a long-range ^1H–^1H COSY experiment in which a long-range coupling was observed between H-12b and H-4, connecting partial structures B and D, while a coupling between H-12b and H-5 connected C and D.



6



6a



6b

The relative stereochemistry of **6** was assigned by difference NOE (3.3–34.9% enhancements) and NOESY experiments. As illustrated in **6b**, the C-4 hydroxyl gave an NOE correlation peak with a hydrogen at C-6, which in turn showed an NOE with the C-12a hydroxyl. NOE correlations were also observed between H-4, Ha-5, and Ha-6 and between Hb-6 and H-12b.

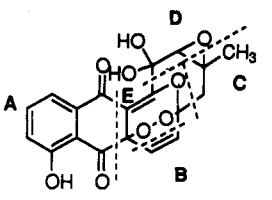
Compound **7** proved to be unstable, and it easily decomposed even at low temperature. A high-resolution mass spectrum could not be obtained, but an *m/z* 386 was observed in the low-resolution EI mass spectrum. With 19 carbons in the ^{13}C NMR spectrum and 14 hydrogens in the ^1H NMR spectrum, a formula of $C_{19}H_{14}O_9$ was indicated for this molecular ion. A DEPT spectrum indicated the presence of one methyl, one methylene, one sp^3 methine, five sp^2 methines, four sp^3 quaternary carbons, and seven sp^2 quaternary carbons (Table 2). The ^1H NMR spectrum of **6** showed the typical ABX spin system of an A ring, one isolated *cis*-double bond at δ 6.18 (d, J = 9.8 Hz) and 6.44 (d, J = 9.8 Hz), and one isolated methylene (AB quartet at δ 2.35 and 2.91, J = 14.5 Hz). A fourth spin system contained a hydroxyl group at δ 4.51 (d, J = 6.5 Hz) coupled to a methine hydrogen at δ 3.78 (d, J = 6.5 Hz). Thus, five partial structures were recognized, which

Table 4. HMBC Connections for Part Structures of 7

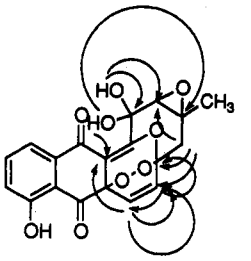
structural fragments	connected by
B and C	H_2–C_4 ($^3J_{CH}$); H_4–C_2 ($^3J_{CH}$); H_{1OH}–C_3 ($^4J_{CH}$); H_2–C_{4a} ($^4J_{CH}$)
B and E	H_{1OH}–C_{12a} ($^4J_{CH}$)
C and D	H_4–C_5 ($^3J_{CH}$)
C and E	H_4–C_{4a} ($^2J_{CH}$)
D and E	H_5–C_{4a} ($^2J_{CH}$); H_6–C_{4a} ($^3J_{CH}$); H_6–C_{12a} ($^3J_{CH}$)

with the molecular formula implied six rings. Only two aryl ketones were observed (δ 196.7 and 185.4); the typical C-1 carbonyl was missing. Six aromatic and olefinic carbons were detected, two of which had attached oxygens (δ 165.8 and 163.2). Resonances at δ 93.0 and 91.0 indicated two carbons that were each bonded to two oxygens.

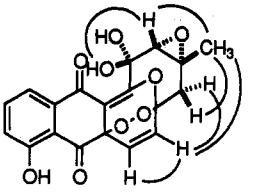
Connections between the partial structures were again obtained from the HMBC spectrum, and these are summarized in **7a**. These data confirmed suspicions that the C-1 carbonyl existed as a hydrate. The key correlations that linked partial structures B–E are given in Table 4. These correlations unambiguously placed the final oxygen as an epoxide bridging C-2 and C-3 (e.g., correlations between H-2/C-3 and H-4/C-2). Difference NOE's (1.5–28.0% enhancements) provided the data for assignment of much of the relative stereochemistry (**7b**). However, it was not possible to define the relationship of the peroxy bridge to the methyl group.



7



7a



7b

Streptomyces are proving quite adept at extensive oxidative modifications of benz[a]anthraquinones.[1] In addition to the proto-typical vicinal C-4a/C-12b diol moiety found in many of these compounds, recent examples include C-6a/C-12a epoxides[20] and a C-6a sulfide/C-12a alcohol.[21] A naphthoquinone modified as a vicinal chlorohydrin has also been reported.[22] However, **6** and **7** appear to be the first naturally occurring seco-derivatives in the angucycline class. The peroxyketal moiety, although rare, is also found in a number of sesquiterpenes.[23,24]

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Table 5. Antibacterial Activities of *Streptomyces phaeochromogenes* Metabolites

organism	MIC (μg/mL) 1	4	5	6	7
Escherichia coli ATCC 10536	8	64	128	>128	>128
Serratia marcescens ATCC 13880	64	64	128	>128	>128
Pseudomonas aeruginosa ATCC 25619	64	128	128	>128	>128
Klebsiella pneumoniae A "AD"[a]	64	64	128	>128	>128
Bacillus subtilis ATCC 6633	8	8	128	>128	>128
Staphylococcus aureus ATCC 25923	8	8	128	>128	>128
Enterococcus faecalis ATCC 29212	4	8	128	>128	>128
Micrococcus luteus ATCC 9341	1	4	128	>128	>128

[a] Obtained from Lederle Laboratories, Pearl River, NY.

Metyrapone appears to have affected the benz[*a*]-anthraquinone metabolic matrix in *S. phaeochromogenes*. Primarily, it decreased accumulation of 1 and increased accumulation of a less oxygenated compound 4. However, 4 would appear to be a shunt product since the original C-5/C-6 double bond has been reduced. This feature is also observed in 6, as well as in previously reported metabolites.[20,21] Since the C-2 hydroxyl of 1 was previously shown to be derived from molecular oxygen,[25] the present result suggests it is introduced through a cytochrome P_{450} reaction. While the C-3 and C-12b hydroxyls of 1 are also derived from molecular oxygen,[25] the biochemical mechanism(s) remain(s) unknown. It cannot be ruled out that they may also involve cytochromes P_{450} since the effect of adding an enzyme inhibitor to a specific fermentation is unpredictable and not necessarily comprehensive.[26]

S. phaeochromogenes WP 3688 is a rich resource of colored metabolites: more than 20 could be observed in extracts of the fermentation broth. The identification of other new metabolites that may be related to 1 is in progress.

Bioactivity

The bioactivities[27] of 4–7 were tested in comparison with 1. The results are shown in Table 5. Only 1, 4, and 5 were active, in this case against Gram-positive bacteria. The novel structures 6 and 7 showed no activity at 128 μg/mL.

Experimental Section

General Procedures. Preparative HPLC was accomplished with two 10-cm, 6-μm Prep NOVA-PAK HR C_{18} columns connected in series and eluted with a mobile phase consisting of CH_3CN–H_2O (30:70 or 22:78; flow rate 5 mL/min; UV detection at 220 nm). UV spectra of metabolites were obtained from the diode array detector for the HPLC.

NMR Studies. ^{13}C chemical shifts are reported in ppm relative to solvent (dioxane-d_8) at 66.5 ppm. HMQC[9] spectra (300 MHz) were obtained with the following acquisition parameters: D_0 = 3 μs, D_1 = 1.0 s, IN = 221 μs, SI_1 = 512 word, SI_2 = 8192 word, SW_1 = 1127 Hz, SW_2 = 14705 Hz; the data were acquired in 256 experiments of 114 scans each. HMBC spectra were obtained using D_0 = 3 μs, D_1 = 2.0 s, D_2 = 3.3 μs, IN = 34 μs, S1 = OH, D_4 = 0.05 s, SI_1 = 512 word, SI_2 = 2048 word, SW_1 = 7267 Hz, SW_2 = 2272 Hz; the data were acquired in 256 experiments of 116 scans each. The ^1H–^1H long-range COSY spectrum (400 MHz) was obtained using D_0 = 3 μs, D_1 = 1.0 s, IN = 454 μs, SI_1 = 512 word, SI_2 = 1024 word, SW_1 = 1101 Hz, SW_2 = 2202 Hz; the data were acquired in 256 experiments of 16 scans each. NOESY spectra were obtained using D_0 = 3 μs, D1 = 2.5 s, IN = 322 μs, SI_1 = 512 word, SI_2 = 1024 word, SW_1 = 1552 Hz, SW_2 = 3105 Hz; the data were acquired in 256 experiments of 42 scans each.

Fermentation, Extraction, and Isolation Procedures. A seed broth (glucose 1.0%, soybean 0.5%, glycerol 0.5%, NaCl 0.3%, $CaCO_3$ 0.3%; pH 7.0, 70 mL in a 250-mL Erlenmeyer flask) was inoculated with a loopful of mycelium from an agar slant of *S. phaeochromogenes* WP 3688. After incubation for 48 h at 28 °C, the seed culture was used to inoculate production broths (same components, 400 mL/2 L Erlenmeyer flask; 1% inoculum). All cultures were grown in a rotary incubator–shaker (1-in. throw) at 28 °C, 250 rpm. Production fermentations (15 L total) were harvested after 80 h by acidification with 1 N HCl to pH 4.0 and removal of the mycelium by filtration. After extraction of the aqueous layer with EtOAc (2 × 7.5 L) the combined organic extracts were dried and concentrated in vacuo to give a brown gum (0.80 g). The residue was worked up in thirds. Each was dissolved in acetone (3 mL) and applied to a column of flash grade SiO_2 (2 × 25 cm, 2% MeOH in $CHCl_3$). A typical profile follows. Elution with 200 mL of 2% MeOH in $CHCl_3$, followed by concentration, gave a fraction containing unknown metabolites (nonpolar oils, 100 mg). Continued elution (300 mL) gave a second fraction (67 mg) containing the components 4–7. The solvent was changed to 4% MeOH in $CHCl_3$ and the first 100 mL discarded. Elution with an additional 400 mL yielded 1 (100 mg). It was important to complete this chromatography within a short period (ca. 30 min) to avoid decomposition. The mixture of 4–7 was further resolved by preparative HPLC (10 mg per injection, elution with 30% CH_3CN in H_2O), and three fractions were collected. The first (~7 mL) was collected beginning at 11.5 min and contained a mixture of 4 and 5. The second (~10 mL) was collected beginning at 14 min and contained 6, while the third (~7 mL) was collected beginning at 17 min and contained 7. Each was extracted with EtOAc, and the extracts were dried and concentrated in vacuo. Both 6 and 7 were rechromatographed under the same conditions, extracted, concentrated, and precipitated from acetone–hexane. The mixture of 4 and 5 (15 mg) was further resolved by preparative HPLC (22% CH_3CN in H_2O, retention times 16 and 19 min, respectively). Combining the pure fractions from each third of the original extract yielded 4 (20 mg), 5 (3.5 mg), 6 (24 mg), and 7 (6 mg) from 15 L of broth.

Compound 4: yellow powder; IR 3413, 1720, 1640, 1615, 1292, 1169 cm^{-1}; UV (CH_3CN–H_2O–HOAc) UV_{max} 216, 242, 264, 440 nm; HR negative FAB MS *m/z* calcd for $C_{19}H_{18}O_7$ 358.1052, found 358.1053; ^1H and ^{13}C NMR see Tables 1 and 2.

Compound 5: pale yellow fluorescent powder; IR 3370, 1751, 1706, 1670, 1455, 1279, 1035 cm^{-1}; UV (CH_3CN–H_2O–HOAc) UV_{max} 236, 265 (sh), 352 nm; HR EI MS *m/z* calcd for $C_{19}H_{16}O_9$ 388.07954 found 388.0793; ^1H and ^{13}C NMR see Tables 1 and 2.

Compound 6: pale yellow fluorescent powder; IR 3399, 2955, 1695, 1635, 1453, 1357 cm^{-1}; UV (CH_3CN–H_2O–HOAc) UV_{max} 236, 352 nm; HR EI MS *m/z* calcd for $C_{19}H_{16}O_8$ 372.0843, found 372.0844; ^1H and ^{13}C NMR see Tables 1 and 2.

Compound 7: pale yellow fluorescent powder; IR 3373, 1807, 1724, 1668, 1454, 1390, 1221, 1136, 871 cm^{-1}; UV (CH_3CN–H_2O–HOAc) UV_{max} 236, 352 nm; EI *m/z* 386 (corresponds to $C_{19}H_{14}O_9$); ^1H and ^{13}C NMR see Tables 1 and 2.

Feeding Experiment with Metyrapone. Metyrapone (452 mg, 2 mmol) in DMSO (3.6 mL) was added dropwise to 5 flasks of *S. phaoechromogenes* WP 3688 (2 L total) 24 h after inoculation. The culture was incubated at 28 °C/250 rpm, harvested after an additional 72 h, and acidified with 1 N HCl to pH 4.0. After filtration, a portion of the aqueous layer (10 mL) was extracted with EtOAc (10 mL). The EtOAc extract was dried and concentrated in vacuo to give a crude sample which was dissolved in MeOH (1 mL) and analyzed by HPLC with photodiode array detection (5-μm NovaPak C_{18} column). This indicated that production of 1 had decreased ca. 10-fold and that of 4 had increased ca. 3-fold (by comparison with a fermentation without adding metyrapone). Workup of the remainder of the fermentation gave 4 (10 mg).

Bioassay for Minimum Inhibitory Concentrations. The minimum inhibitory concentrations were determined by the

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microdilution method as prescibed in ref 27. The compounds were dissolved in MeOH.

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of 4–7 (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.