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ISOLATION AND SYNTHESIS OF BENZ[*a*]ANTHRAQUINONES RELATED TO ANTITUMOR AGENT PD116740

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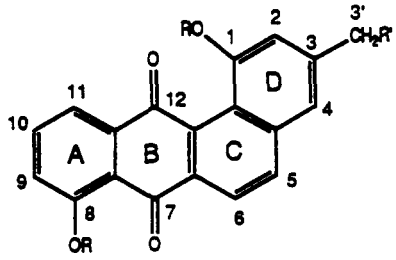
ABSTRACT.—Two new benz[*a*]anthraquinones **1** and **2** and two known benz[*a*]anthraquinones **3** and **4** have been isolated as minor components from fermentations of actinomycete WP4669. Structural elucidation was based on standard spectroscopic analysis of compounds **1** and **2** and their acetates **5** and **6**. Confirmation of structural assignments was achieved through synthesis of **1** and **2** from PD116740 [**7**]. Structural elucidation of minor products **8** and **9** and their acetates **10** and **11** produced from this synthetic route is also discussed.

Benz[*a*]anthraquinone PD116740 [**7**], isolated from an unidentified actinomycete isolate, WP4669 (1), exhibits activity against L1210 lymphocytic leukemia and HCT human colon adenocarcinoma cell lines. PD116740 is interesting among the rapidly growing benz[*a*]anthraquinone family of antitumor antibiotics in that it possesses a benzyl alcohol moiety in the D ring and a unique dihydrodiol oxidation pattern in the C ring. Recent biosynthetic investigations (2–4) have shown that the angular benz[*a*]anthraquinone skeleton is acetate-derived via a single decaketide precursor, although biosynthesis of benz[*a*]anthraquinone PD116198 has been reported to proceed through a novel rearrangement of a linear tetracyclic structure (5). A benz[*a*]anthraquinone has also been proven to be an intermediate in the biosynthetic pathway of the kinamycin antibiotics through oxidative cleavage and rearrangement of the C ring of dehydrorabelomycin (6). It has also been proposed that biosynthesis of the gilvocarcin family of antitumor antibiotics may require oxidative cleavage of the C ring of a benzanthraquinone or benzanthracene precursor (7). PD116740 [**7**] is biosynthetically interesting because a unique 5,6-dihydroxy functionality is present in its C ring. During our preliminary investigations into the biosynthesis of PD116740, two new benz[*a*]anthraquinone derivatives **1** and **2** were isolated, along with the known benz[*a*]anthraquinones tetrangulol [**3**] and tetrangomycin [**4**] (8,9). Confirmation of proposed structures for **1** and **2** has been accomplished by their synthesis from PD116740. Structural elucidation of two minor products **8** and **9** produced in the synthesis of **1** and **2** is also discussed.

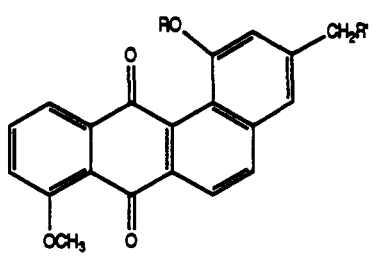
RESULTS AND DISCUSSION

The major metabolite of actinomycete WP4669 is PD116740 [**7**]. Chromatographic separation of the many minor components present in extracts of WP4669 cells first yielded two nonpolar brown products. The less polar product had a ^{1}H nmr identical to that reported for tetrangulol [**3**] (8,9). All physical and spectral data for both the isolated compound and its acetate **12** (^{1}H-nmr data is tabulated in Table 1) were identical to those reported for tetrangulol and its diacetate. Tetrangulol was first reported as a dehydration product of tetrangomycin [**4**] (8), although it was later isolated from fermentation cultures of *Streptomyces rimosus* (9). Tetrangomycin is also present at low concentrations in broths of WP4669 fermentations. The ^{1}H-nmr spectrum of tetrangomycin fractions isolated from the broth of WP4669 fermentations matched that reported for tetrangomycin in 1965 (8). Additionally, tetrangomycin was converted to tetrangulol [**3**], by mild treatment with aqueous NaOH, as originally described for tetrangomycin in 1966 (9).

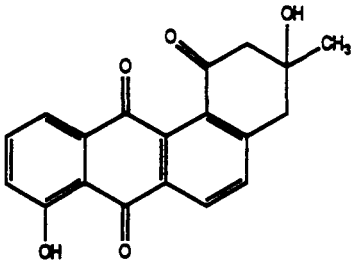
The second nonpolar brown product isolated from cell extracts of WP4669 fermentations also had a ^{1}H-nmr spectrum very similar to that of tetrangulol [**3**]. Two H-



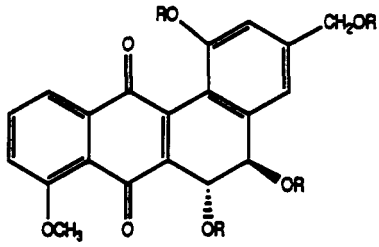
1 R=H, R′=Cl
3 R=R′=H
5 R=Ac, R′=Cl
12 R=Ac, R′=H
14 R=H, R′=OH



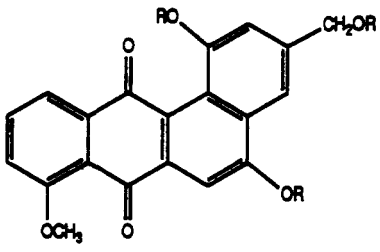
2 R=H, R′=OH
6 R=Ac, R′=OAc
9 R=H, R′=Cl
11 R=Ac, R′=Cl



4



7 R=H
13 R=Ac



8 R=H
10 R=Ac

bonded phenolic signals were present at δ 12.19 and 11.45, and the aromatic region displayed an AB quartet at δ 8.41 and 8.22, characteristic of benz[*a*]anthraquinones with unsubstituted C rings. However, the benzylic methyl signal of tetrangulol [**3**] had been replaced by a two-proton singlet at δ 4.71, suggesting a substituted benzyl moiety in the second nonpolar metabolite. Acetylation of this new isolate gave a diacetate, by ^{1}H-nmr analysis (see **5**, Table 1). Fabms of the diacetate showed an $[M+1]^+$ peak at *m/z* 423 and an $[M+3]^+$ peak at *m/z* 425. The ratio of the $[M+1]^+$ to $[M+3]^+$ peaks was 3 to 1, characteristic of chlorine-containing compounds. The diacetate has a molecular formula of $C_{23}H_{15}ClO_6$, as determined by hrms. The above data, as well as the uv, ir, and ^{13}C-nmr data, are consistent with proposed structure **1**, chlorotetrangulol, for the natural product, and **9** for its diacetate. Chlorotetrangulol [**1**] has also been synthesized from PD116740 tetraacetate [**13**] in two steps, as described below.

TABLE 1. ^{1}H-nmr Data (in δ) of Benz[*a*]anthraquinones **12**, **5**, **6**, **10**, and **11**.[a]

Proton	Compound				
	12 (R′=H)	**5** (R′=Cl)	**6** (R′=OAc)	**10** (R′=OAc)	**11** (R′=Cl)
H-2	7.31 br s	7.41 d (1.5)	7.52 d (1.5)	7.52 d (1.5)	7.52 d (1.8)
H-4	7.55 br s	7.81 d (1.5)	7.84 d (1.5)	7.86 br s	7.81 d (1.8)
H-5	8.02 d (8.1)	8.12 d (8.8)	8.19 d (8.4)	—	8.12 d (8.5)
H-6	8.19 d (8.1)	8.27 d (8.8)	8.40 d (8.4)	8.11 s	8.21 d (8.5)
H-9	7.39 dd (1.5, 8.1)	7.41 d (8.1)	7.36 d (9.6)	7.3 d (8.0)	7.31 d (7.2)
H-10	7.77 t (8.1)	7.81 t (8.1)	7.73–7.81 m	7.72 t (8.0)	7.64–7.76 m
H-11	7.98 dd (1.5, 8.1)	8.01 d (8.1)	7.73–7.81 m	7.65 d (8.0)	7.64–7.76 m
1-OAc	2.50 s	2.51 s	2.45 s	2.39 s	2.39 s
3-CH_2R'	2.54 s	4.76 s	5.35 s	5.29 s	4.76 s
3-R′	—	—	2.22 s	2.16 s	—
5-OAc	—	—	—	2.53 s	—
8-OAc	2.37 s	2.38 s	—	—	—
8-OMe	—	—	4.12	4.06 s	4.06 s

[a]Data recorded at 300 MHz in $CDCl_3$; figures in parentheses are *J* in Hz.

Attempts to deacetylate PD116740 tetraacetate [**13**] (1) using mild base catalysis resulted in formation of two new products. These two products were also recovered from direct reaction of PD116740 [**7**] in aqueous NaOH, although in much lower yields. Reaction of **7** and **13** with aqueous acid also produced the same two components, along with a number of partially acetylated products. The major product in all of these reactions was dark brown in color and the minor product was purple.

Comparison of ^{1}H-nmr data of this new brown product with that of chlorotetrangulol [**1**] revealed that the two spectra differed only in that an H-bonded phenolic signal of **1** had been replaced by a methoxy signal at δ 4.00 in the spectrum of the new product. Acetylation of the new product resulted in formation of a diacetate. In the ^{1}H-nmr spectrum of the diacetate (see **6**, Table 1) the methylene singlet of the original brown product had shifted from δ 4.90 to 5.35 in the diacetate, suggesting acetylation of a benzyl alcohol functionality, as is present in the starting material **13**. Except for the methoxy singlet at δ 4.12, the remainder of the ^{1}H-nmr spectrum, including the AB quartet in the aromatic region, of the diacetate of the new brown product is very similar to the spectrum of chlorotetrangulol [**1**]. Thus the new brown product was assigned structure **2**, and its diacetate was assigned structure **6**. ^{13}C-nmr, ir, uv, and hrms data are all consistent with these assignments.

The minor product produced from aqueous NaOH treatment of **13** was bright purple and only slightly soluble in most solvents. A ^{1}H-nmr spectrum could be obtained only in DMSO-d_6 and was similar to the spectrum of **2**, in that a methoxy signal at δ 3.93 ppm, a methylene signal at δ 4.62, and an H-bonded phenolic signal at δ 11.52 were present. In contrast, the aromatic region of the purple product was lacking the AB quartet, indicating substitution in the C ring. Acetylation of the purple product gave a triacetate (see **10**, Table 1), implying that in addition to the H-bonded phenol and the benzylic alcohol, a third alcohol functionality was present, perhaps a C-ring phenol. As there was only one hydrogen-bonded phenolic proton (due to the C-1 phenolic proton) in the ^{1}H-nmr spectrum of the purple product **8**, the phenol must be at C-5, rather than at C-6 as in dehydrorabelomycin (6). Assignment of the phenol at C-5 has been confirmed by 2D nmr experiments on the triacetate **10**. In the lr HETCOSY spectrum

of **10** there is correlation between the C-ring proton at δ 8.11 and the C-7 carbonyl at 180.6 ppm. There was no correlation between the proton at δ 8.11 with C-4 at 123.9 ppm, eliminating the possibility that the phenol is on C-6. Additionally, there is correlation between H-4 at δ 7.86 and the phenolic C-5 at 150.8 ppm. All other correlations in the lr HETCOSY spectrum were consistant with proposed structure **10** for the purple triacetate.

From a biosynthetic perspective, both new benz[*a*]anthraquinones **2** and **8** are potential precursors to PD116740 [7]. Although **2** has been isolated from fermentation broths of WP4669, its presence as a natural metabolite, rather than as a decomposition product of **5**, has not yet been confirmed. In contrast to the brown product **2**, the purple product **8** has not been detected in fermentation broths of WP4669. Biosynthetic investigations with these products are in progress.

Attempts to produce a potential biosynthetic intermediate **14** by acid-catalyzed hydrolysis of **2** resulted in the formation of two very nonpolar brown products. Tlc analysis of the reaction mixture of **2** in refluxing 6 M HCl in CF_3CO_2H showed that a new nonpolar product formed initially and was slowly converted to an even less polar product. The ^{1}H-nmr spectrum of the product formed initially was very similar to that of **2**. It retained both the methoxy signal at δ 4.09 and a two-proton benzylic singlet at δ 4.71. Acetylation gave a monoacetate by ^{1}H-nmr analysis (see **11**, Table 1). Hrms analysis of the acetate gave a molecular formula of $C_{22}H_{15}ClO_5$, which is consistent with proposed structure **11** for the acetate and **9** for the original synthetic product.

The slower forming, less polar product in the reaction mixture had an R_f identical to that of chlorotetrangulol [**1**]. The ^{1}H-nmr, ir, and uv spectra of **1** and the synthetic product were found to be identical in every respect. Additionally, acetylation of the new product gave a diacetate which was identical to **5** in every respect. Thus, treatment of **2** with HCl in CF_3CO_2H initially resulted in substitution of the benzylic hydroxy group with chlorine to give **9**. Continued reaction finally resulted in hydrolysis of the C-8 methoxide to give **1**. In contrast to chlorotetrangulol [**1**], compound **9** has not been detected in the broth or cell extracts of WP 4669 fermentations by tlc analysis.

The similarity of natural metabolites **1**, **2**, and **3** to PD116740 [7], the major metabolite of WP4669 fermentations, suggests that they may play a role in the biosynthesis of **7**. Biosynthetic studies with these metabolites are currently in progress, as is investigation of the bioactivity of these new natural and synthetic benz[*a*]anthraquinones.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined in a Meltemp capillary melting point appratus are are uncorrected. Ir spectra were obtained on a Mattson Polaris™ Ft-ir spectrophotometer, and uv spectra were obtained on an HP 8451A Diode Array spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were taken on either a Jeol FNM-FX90Q or a GN300 (at North Carolina State University). Two-dimensional nmr experiments were done on a GN500 instrument (at North Carolina State University). Nmr spectra are reported as ppm downfield from TMSi. Hrfabms [thioglycerol-glycerol (30:70) matrix] data were obtained on a VG ZAB-E spectrometer (at Research Triangle Institute).

Tlc was done on Merck precoated Si gel 60 PF-254 plates, 0.25 m thick. Cc was done on flash grade SiO_2 (Fisher) or Amberlite XAD-2 resin (Sigma). Fermentations were carried out in a Lab Line Model 3525 gyrotory incubator shaker. Broths were centrifuged in a IEC Model PR-6 centrifuge. A culture of actinomycete 4669 was a gift from the culture collection of Dr. Steven Gould, Department of Chemistry, Oregon State University, Corvallis, OR. All chemicals were of reagent grade.

FERMENTATION AND ISOLATION.—Actinomycete 4669 was maintained at 4° on agar slants composed of 1% corn starch, 0.2% NZ-Amine (Sigma), 0.1% beef extract, 0.1% yeast extract, and 2.0% agar (pH 7.2). Seed cultures were prepared by inoculating 100 ml of medium containing 2% glucose, 0.3% $CaCO_3$, 0.3% NaCl, and 0.5% corn steep liquor with growth from an agar slant. The cultures, contained in 500-ml Erlenmeyer flasks, were incubated at 24–26° at 250 rpm for 68 to 72 h. Production broths [200

ml of 1.0% glycerol, 1.0% Monkey Chow (Purina #5045) adjusted to pH 7.0, in 1-liter Erlenmeyer flasks] were inoculated with 5.0 ml from the seed culture. The production cultures were incubated at 24–26° at 250 rpm for 92 h.

Broth from a 92 h, 1.6-liter fermentation of actinomycete 4669 was transferred to centrifuge bottles, and the cells were pelleted by centrifugation (5000 rpm, 10 min). The supernatant was decanted and the cell pellets were resuspended in H_2O (200 ml) and recentrifuged (5000 rpm, 10 min). The supernatants were combined for chromatography through Amberlite XAD-2 resin. The cell pellets were soaked in Me_2CO (200 ml) and after 2–3 h were filtered through a celite pad. The celite pad was then washed with Me_2CO (400 ml), and the resulting filtrate was concentrated to give 0.76 g of a dark brown gum.

The broth supernatant was passed through an Amberlite XAD-2 column (300 ml in H_2O). After washing with H_2O (300 ml), PD116740 [**7**] plus traces of other secondary metabolites was eluted with MeOH (600 ml), which after concentration gave 0.85 g of crude orange extract. Flash cc of the concentrate [SiO_2, CH_2Cl_2-MeOH (9:1)] first afforded the less polar metabolites, followed by PD116740 [**7**]. Fractions containing pure **7** were combined, concentrated, and recrystallized in EtOAc to give 335 mg of **7**. Spectral data for **7** were identical to those reported in the literature (1). Fractions containing the nonpolar metabolites were combined with the cell extract. The resulting concentrate was subjected to flash cc (SiO_2, increasing concentrations of MeOH in CH_2Cl_2). Elution with CH_2Cl_2-MeOH (99:1) first gave oily dark brown fractions containing the nonpolar metabolites **3** and **1** (262 mg). Elution with CH_2Cl_2-MeOH (95:5) gave fractions containing **2** and **4** (23 mg).

The nonpolar metabolites **3** and **1** were further purified by radial chromatography [1 mm SiO_2 plate, hexanes-EtOAc (6:1)]. Tetrangulol [**3**] eluted first, followed by chlorotetrangulol [**1**]. Pure fractions of each were concentrated and crystallized from CH_2Cl_2/hexanes. Fractions containing **2** and **4** were further purified by radial chromatography [1 mm SiO_2, CH_2Cl_2-Et_2O (3:1)]. Tetrangomycin [**4**] eluted first, followed by the brown product **2**. Pure fractions of each were concentrated.

Tetrangulol [**3**] crystallized as dark brown needles (5.6 mg per 1.6 liter broth): mp 197.5–199° [lit. (9) mp 199–201]; uv λ max (CH_2Cl_2) 214, 234, 320, 432 nm; ir ν max (KBr) 1644, 1478, 1453, 1310, 1293, 1254, 1217, 1207, 1158, 792 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ 12.26 (s, 1H), 11.27 (s, 1H), 8.35 (1H, d, J = 8.8 Hz), 8.15 (1H, d, 8.8 Hz), 7.59 (1H, brs), 7.26–7.90 (3H, m), 7.59 (1H, brs), 2.46 (3H, s).

Chlorotetrangulol [**1**] (2.3 mg per 1.6 liter broth), crystallized as dark brown needles: mp 204–205°; uv λ max (CH_2Cl_2) 234, 318, 434 nm; ir ν max (KBr) 3440, 1636, 1617, 1489, 1454, 1442, 1414, 1297, 1271, 1260 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ 12.19 (1H, s), 11.45 (1H, s), 8.41 (1H, d, J = 8.5 Hz), 8.22 (1H, d, 8.5 Hz), 7.38–7.95 (3H, m), 7.48 (1H, brs), 7.30 (1H, brs), 4.71 (2H, s).

Tetrangomycin [**4**] was recovered as a yellow film (3.5 mg per 1.6 l broth): ir ν max (film) 3362, 2903, 1765, 1718, 1696, 1684, 1456, 1454, 1366, 1218 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ 12.3 (1H, s), 7.33–7.71 (m, 5H), 3.18 (2H, br s), 3.08 (2H, d, J = 7.7 Hz), 1.53 (3H, s).

Compound **2** crystallized from CH_2Cl_2/hexanes as brown needles (3.0 mg per 1.6 l broth): mp 222–223°; uv λ max (EtOH) 210, 232, 314, 412 nm; ir (KBr) ν max 3500, 2920, 1657, 1584, 1436, 1414, 1406, 1269, 1032 cm^{-1}; ^1H nmr (90 MHz, $CDCl_3$) δ 11.26 (1H, s), 8.35 (1H, d, J = 8.8 Hz), 8.21 (1H, d, J = 8.8 Hz), 8.08 (1H, dd, J = 7.1, 0.8 Hz), 7.75 (1H, t, J = 7.1 Hz), 7.26–7.47 (3H, m), 4.88 (2H, brs), 4.09 (3H, s).

PREPARATION OF ACETATES **12**, **5**, AND **6**.—*Tetrangulol diacetate* [**12**].—Tetrangulol [**1**] isolated from cells was pooled (20 mg) and treated with Ac_2O (0.5 ml) in pyridine (2.0 ml) at room temperature for 2 h. Excess reagent was removed in vacuo, and the yellow residue was chromatographed [flash SiO_2, CH_2Cl_2-MeOH (99:1)]. Crystallization from Et_2O/hexanes gave **12** (18 mg, 70.5%) as yellow needles: mp 174–176° with solidification and remelting at 182–183° [lit. (9) mp 177–178° with solidification and remelting at 186–187°]; uv λ max (CH_2Cl_2) 222, 244, 296, 338 nm; ir ν max (KBr) 1769, 1668, 1594, 1448, 1365, 1281, 1262, 1202, 1105, 1010 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr (75 MHz, $CDCl_3$) 184.9, 181.3, 169.5, 168.9, 149.5, 147.1, 140.1, 137.9, 135.0, 134.3, 133.8, 133.2, 128.7, 125.9, 125.7, 124.4, 123.8, 122.3, 120.9, 21.5, 21.1, 21.0 ppm.

Chlorotetrangulol acetate [**5**].—Chlorotetrangulol [**1**] isolated from cells was pooled (12.8 mg) and acetylated as described above. Crystallization from Et_2O/hexanes, gave **5** (11 mg, 69%) as yellow needles: mp 204–205°; uv λ max (CH_2Cl_2) 220, 290, 336, 396 nm; ir ν max (KBr) 1775, 1671, 1667, 1596, 1366, 1281, 1262, 1191, 1170, 1104 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr (75 MHz, $CDCl_3$) 184.8, 180.9, 169.3, 168.4, 149.6, 148.1, 138.5, 137.5, 134.9, 134.3, 133.3, 128.7, 126.1, 125.7, 125.4, 124.2, 123.8, 123.6, 122.6, 122.4, 44.8, 20.9, 20.8 ppm; fabms *m/z* (%) $[M + 1]^+$ 423 (100), 425 (32), 382 (45), 381 (82), 340 (68), 339 (88), 279 (43), 130 (70); hrfabms $[M + 1]^+$ *m/z* 423.0659 (calcd for $C_{23}H_{15}ClO_6$, 423.0635).

3′-Hydroxy-8-methoxytetrangulol diacetate [**6**].—Compound **2** (12.5 mg) isolated from cells (or synthe-

sized from **13** as described below) was acetylated and purified as described above. Crystallization from Et_2O/hexanes gave **6** (13.1 mg, 84%) as yellow needles: mp 218.5–220°; uv λ max (EtOH) 218, 286, 384, 656 nm; ir ν max (KBr) 1631, 1585, 1470, 1367, 1288, 1271, 1250, 1234, 1190, 975 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr (75 MHz, $CDCl_3$) 185.6, 181.6, 170.8, 168.6, 159.5, 147.4, 138.1, 137.1, 137.0, 135.5, 135.2, 134.0, 132.7, 124.9, 123.0, 122.9, 122.1, 120.8, 118.3, 116.7, 64.9, 56.3, 20.8, 20.7 ppm; fabms *m/z* (%) $[M+1]^+$ 419 (100), 420 (65), 378 (33), 377 (36), 317 (21), 279 (52), 201 (22), 93 (32); hrfabms *m/z* $[M+1]^+$ 419.1154 (calcd for $C_{24}H_{18}O_7$, 419.1131).

SYNTHESIS OF COMPOUNDS **1**, **2**, **8**, AND **9**.—PD116740 tetraacetate (1) [**13**] (40 mg) was dissolved in 2 ml warm MeOH, 1 M NaOH (10 ml) was added, and the mixture was heated for 1 h at 50°. After cooling to room temperature, the mixture was acidified with 1 M HCl and extracted with EtOAc (3 × 25 ml). The combined organic phases were dried (anhydrous $MgSO_4$) and concentrated. Compounds **2** and **8** were separated by radial chromatography [1 mm SiO_2, CH_2Cl_2-MeOH, (9:1)]. The brown product **2** eluted first and crystallized from CH_2Cl_2/hexanes as brown needles (16.5 mg, 66%). Physical and chemical data for synthetic **2** are identical to those reported above for **2** isolated from fermentations. The purple product **8** eluted next and crystallized from EtOAc/hexanes as dark purple needles (3.3 mg, 13%): mp >260°; uv λ max (EtOH), 234, 288, 334, 582 nm; ir ν max (KBr) 3392, 3295, 1656, 1515, 1470, 1455, 1437, 1317, 1293, 1248 cm^{-1}; ^1H nmr (90 MHz, DMSO-d_6) δ 11.52 (1H, s), 7.5–7.8 (5H, m), 7.07 (1H, brs), 4.62 (2H, brs), 3.93 (3H, s).

Compound **2** (28 mg) was dissolved in 5 ml CF_3COOH and 5 ml 6 M HCl and refluxed for 24 h. After cooling to room temperature, H_2O (50 ml) was added and the resulting mixture was extracted with CH_2Cl_2 (3 × 25 ml). The combined organic phases were dried (anhydrous $MgSO_4$) and concentrated. Compounds **1** and **9** were separated by radial chromatography [1 mm SiO_2, hexanes/CH_2Cl_2, (2:1)]. Chlorotetrangulol [**1**] eluted first and crystallized from CH_2Cl_2/hexanes as brown needles (6.4 mg, 23%): mp 204–205° (**1** isolated from WP4669 cells: mp 202–206°). All spectral data for synthetic **1** were identical to those reported above for **1** isolated from fermentations. The more polar product **9** eluted next and crystallized from CH_2Cl_2/hexanes as dark brown needles (3.5 mg, 12%): mp 218.5–220°; uv λ max (EtOH), 200, 232, 312, 414 nm; ir ν max (KBr) 3500, 1648, 1593, 1564, 1422, 1269, 1026, 961, 782, 691 cm^{-1}; ^1H nmr (90 MHz, DMSO-d_6) δ 11.34 (1H, s), 8.38 (1H, d, J = 8.5 Hz), 8.20 (1H, d, J = 8.5 Hz), 7.30–7.95 (5H, m), 4.71 (2H, s), 4.09 (3H, s).

PREPARATION OF ACETATES **10** AND **11**.—The purple product **8** (7.7 mg) was treated with Ac_2O (0.5 ml) in pyridine (2.0 ml) at room temperature for 2 h. Excess reagent was removed in vacuo, and the yellow residue was chromatographed [flash SiO_2, CH_2Cl_2-MeOH (99:1)]. Crystallization from Et_2O/hexanes gave **10** (6.0 mg, 57%) as yellow needles: mp 217.5–220°; uv λ max (CH_2Cl_2) 218, 284, 388 nm; ^1H nmr see Table 1; ^{13}C nmr (125 MHz, $CDCl_3$) 184.9, 180.6, 170.6, 168.6, 168.3, 159.8, 150.8, 147.8, 138.2, 137.7, 136.2, 135.5, 131.1, 130.8, 124.2, 123.9, 120.2, 118.5, 118.4, 116.9, 115.4, 65.2, 56.5, 21.1, 21.0, 20.9 ppm; fabms *m/z* (%) $[M+1]^+$ 477 (100), 478 (68), 436 (25), 435 (23), 419 (28), 394 (13), 393 (14), 280 (13), 217 (13), 192 (12); hrfabms *m/z* $[M+1]^+$ 477.1185 (calcd for $C_{26}H_{20}O_9$, 477.1186).

Compound **9** (8.5 mg) was acetylated as described above. Crystallization from Et_2O/hexanes gave **11** (7.5 mg, 79%) as yellow needles: mp 164.5–165.5°; uv λ max (CH_2Cl_2) 218, 238, 286, 384 nm; ir ν max (KBr) 1781, 1100, 1183, 1253, 1273, 1293, 1363, 1587, 1673, 1781 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr (75 MHz, $CDCl_3$) 184.5, 181.6, 168.7, 159.8, 147.5, 138.3, 138.2, 137.3, 135.8, 135.4, 134.2, 133.0, 125.6, 123.7, 123.2, 122.5, 118.5, 116.9, 115.2, 56.5, 45.0, 20.9 ppm; fabms *m/z* (%) $[M+1]^+$ 495 (100), 397 (47), 354 (48), 353 (44), 312 (22); hrfabms *m/z* $[M+1]^+$ 395.0683 (calcd for $C_{22}H_{15}ClO_5$, 395.0686).

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