Biosynthesis of PD 116740: Origins of the Carbon, Hydrogen, and Oxygen Atoms and Derivation from a 6-Deoxybenz[*a*]anthraquinone

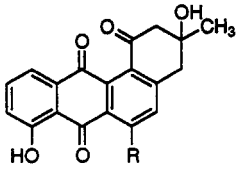
Steven J. Gould,* Xing-chun Cheng, and Chris Melville

Contribution from the Department of Chemistry, Oregon State University, Corvallis, Oregon 97331-4003

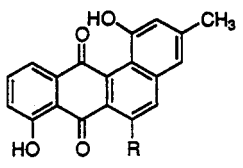
Received September 27, 1993®

Abstract: The benz[*a*]anthraquinone antibiotic PD 116740 is formed from the regular cyclization of a decaketide intermediate folded in a manner to generate the angular tetracyclic skeleton. The 6-deoxybenz[*a*]anthraquinone tetrangulol is an intermediate, indicating that 6-deoxygenation occurs at a prearomatic stage in the biosynthesis. This was consistent with the lack of incorporation of acetate-derived oxygen at this site. Labelling of the C-5 hydroxyl by molecular oxygen indicates that enzymatic epoxidation of the K-region double bond, followed by action of an epoxide hydrolase, generates the 5,6-*trans*-diol moiety.

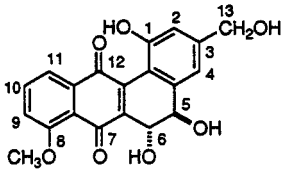
Benz[*a*]anthraquinones (isotetracenones,[1] angucyclinones[2]) have in recent years become a major class of aromatic polyketide metabolites. Many of them have a variety of potent biological activities. Of those so far studied, all but one are derived biosynthetically from the predictable folding of a decaketide precursor.[3-7] In considering potential early branch points leading to modification of the fundamental skeleton, the presence or absence of an oxygen function at C-6 was viewed to be a significant feature. The simplest representatives of 6-oxobenz[*a*]anthraquinones are rabelomycin (**1**)[8] and dehydrorabelomycin (**2**),[4] while



1, R = OH
3, R = H



2, R = OH
4, R = H



5

the simplest representatives lacking this function are tetrangomycin (**3**)[9,10] and tetrangulol (**4**).[9,10] We have recently established that the hydroxyl oxygen at C-6 of **2** is derived from the original acetate precursor, while the corresponding oxygen in the antibiotic PD 116740 (**5**) is not.[7] Furthermore, **4** rather than **2** is an intermediate in the latter pathway.[7] We now report further details on the biosynthesis of **5** and the implications of these findings for a number of other biosynthetic pathways.

Table 1. ^{13}C NMR Spectrum of PD 116740 and Incorporation of Labelled Precursors

			precursor[a]			
carbon	chemical shift[b] (δ)	J_{CC} (Hz)	**3b + 3c** coupled pair	**3d** $\Delta\delta$	**3e** $\Delta\delta$	$^{18}O_2$ $\Delta\delta$
1	157.3	70.6	a	0.01		
2	117.0					
3	147.5	46.8	b		0.06, 0.13	
4	121.3	62.4	c			
4a	140.4	62.0	c			
5	72.7	42.9	d			0.02
6	64.8	43.1	d			
6a	142.2	53.8	e			
7	184.2	53.9	e			
7a	120.5	70.6	f			
8	160.6	70.6	f	0.02		
9	120.1	60.5	g			
10	137.5	60.5	g		0.07	
11	119.2	56.9	h			
11a	136.5	56.9	h		0.10	
12	187.2	52.6	i			
12a	142.9	52.6	i			
12b	115.6	70.7	a			
13	64.4	46.8	b			

[a] Sites of enrichment indicated by J_{CC} coupling constants or by isotope-induced shifts of δ (in ppm). [b] DMSO-d_6; 100.6 MHz; spectral width 25 000 Hz; 128K data points; 2.8-s acquisition time; 35° pulse; 16 000–39 000 scans.

Results

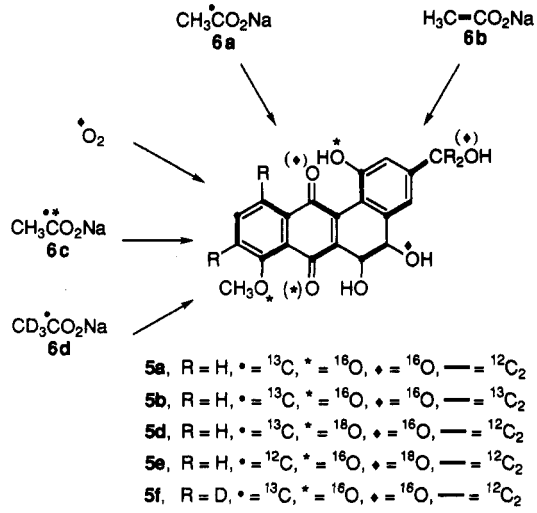
PD 116740, produced by *Streptomyces* WP 4669, is active against HCT-8 human adenocarcinoma and L1210 lymphocytic leukemia.[11] Its structure was determined by a single-crystal X-ray diffraction analysis, which also established the *trans* relative stereochemistry of the diol moiety. In preparation for biosynthetic studies, we obtained the ^{13}C NMR assignments (Table 1) through a series of 1D and 2D NMR experiments. *S.* WP 4669 was then grown in liquid culture, and a mixture of sodium [2-^{14}C]acetate and sodium [1-^{13}C]acetate (**6a**) was fed in three pulses, beginning

* Abstract published in *Advance ACS Abstracts,* February 15, 1994.

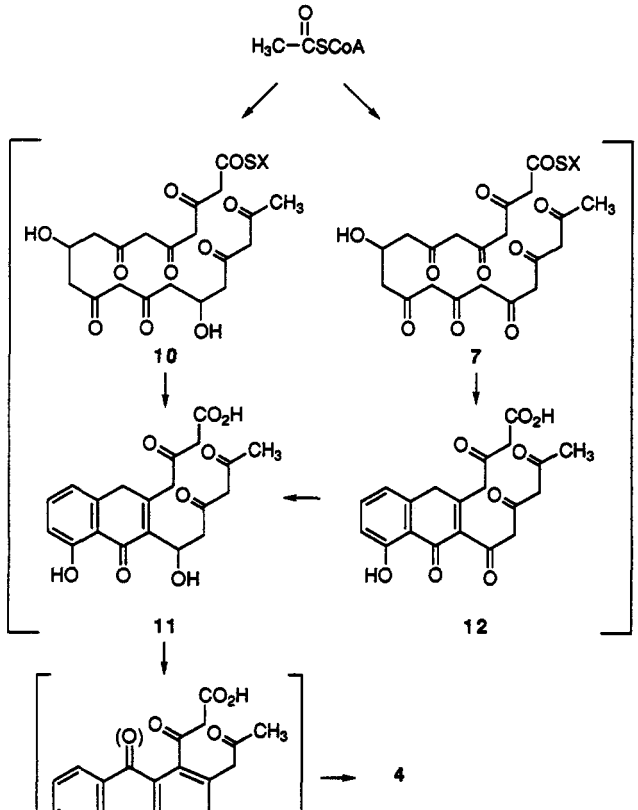
(1) Hayakawa, Y.; Ha, S.-C.; Kim, Y. J.; Furihata, K.; Seto, H. *J. Antibiot.* **1991**, *44*, 1179–1186.
(2) Rohr, J.; Thiericke, R. *Natl. Prod. Rep.* **1992**, *9*, 103–137.
(3) Imamura, N.; Kakinuma, K.; Ikekawa, N.; Tanaka, H.; Omura, S. *J. Antibiot.* **1982**, *35*, 602–608.
(4) Seaton, P. J.; Gould, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 5282–5284.
(5) Rohr, J.; Beale, J. M.; Floss, H. G. *J. Antibiot.* **1989**, *42*, 1151–1157.
(6) Gould, S. J.; Halley, K. A. *J. Am. Chem. Soc.* **1991**, *113*, 5902–5903.
(7) Gould, S. J.; Cheng, X.-c.; Halley, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 10066–10068.
(8) Liu, W.-C.; Parker, W. L.; Slusarchyk, D. S.; Greenwood, G. L.; Graham, S. F.; Meyers, E. *J. Antibiot.* **1970**, *XXIII*, 437–441.
(9) Dann, M.; Lefemine, D. V.; Barbatschi, F.; Shu, P.; Kunstmann, M. P.; Mitscher, L. A.; Bohonos, N. *Antimicrob. Agents Chermother.* **1965**, 832–835.
(10) Kuntsmann, M. P.; Mitscher, L. A. *J. Org. Chem.* **1966**, *31*, 2920–2925.
(11) Wilton, J. H.; Cheney, D. C.; Hokanson, G. C.; French, J. C. *J. Org. Chem.* **1985**, *50*, 3936–3938.

0002-7863/94/1516-1800$04.50/0

Scheme 1

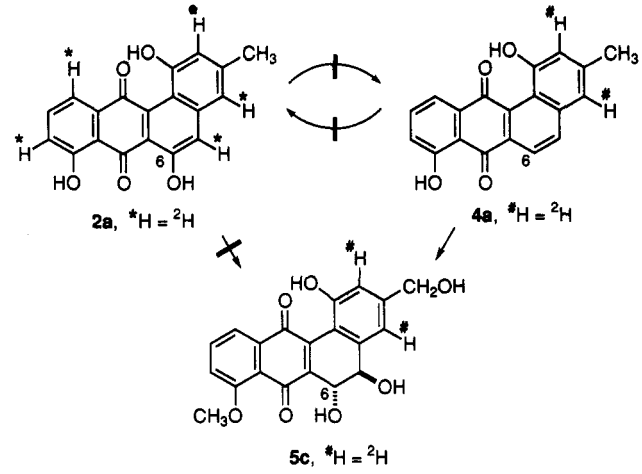


Scheme 2

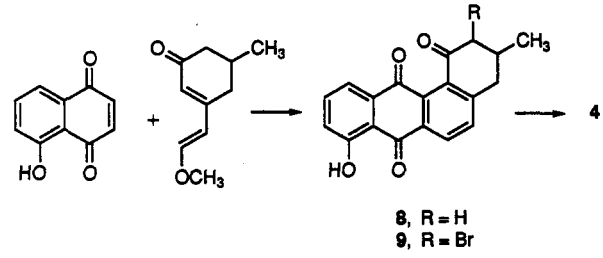


at the onset of the production of **5** (as detected by tlc analysis). After a total fermentation time of 72 h, purification by chromatographies on Diaion HP-20 and silica gel, followed by recrystallization, yielded pure **5a** (Scheme 1). Liquid scintillation analysis of a sample indicated a 0.4% incorporation of acetate. Analysis by ^{13}C NMR spectroscopy revealed the expected nine enriched resonances (average enrichment = 5.5%). Sodium [1,2-$^{13}C_2$]acetate (**6b**) was fed next in order to establish the nature of the biosynthetic backbone, and this yielded **5b**. The ^{13}C NMR spectrum showed eighteen resonances with doublets flanking the natural abundance singlets, indicating the incorporation of nine intact precursor acetate units and one lone enriched singlet; however, the correct pairings could not be made directly due to the similarity of four coupling constants. A 2D INADEQUATE

Scheme 3



Scheme 4



experiment clarified the situation, and the proper pairings were made (Table 1). This revealed the folding of the precursor polyketide chain **7** or **10** as shown in Scheme 2, which is the expected pattern for a regular benz[*a*]anthraquinone.

A sample of [2,4,5,9,11-$^2H_5$]dehydrorabelomycin (**2a**), a compound previously incorporated into kinamycin D,[4] was now fed to *S.* WP 4669. Although **2** could no longer be detected in the broth upon termination of the fermentation, ^2H NMR analysis of the **5** produced in this experiment showed no deuterium enrichment (Scheme 3).

In conjunction with another project, we had developed an efficient synthesis of **4** (Scheme 4) that allowed an expeditious test of it as an alternative intermediate. Thus, ochromycinone (**8**) was prepared with minor modifications of the sequence reported by Guignant.[12] Compound **8** was then brominated at C-2 to yield **9**, which was dehydrobrominated with lithium bromide and lithium carbonate in DMF. The yield of **4** for the two steps was 80–90%. Deuterium exchange using the conditions previously established for preparing **2a**—deuteriotrifluoroacetic acid at 110–120 °C under a nitrogen atmosphere for two days—gave a 98% yield of **4a**. Surprisingly, only H-2 and H-4 were exchanged (97%); H-9 and H-11 were unchanged. In two separate experiments, a sample of **4a** was introduced to *S.* WP 4669 using the standard feeding protocol. Each time, workup afforded **5c**, along with recovery of approximately half the material that had been fed (essentially unchanged in deuterium content). ^2H NMR analysis of **5c** clearly showed overlapping resonances at δ 6.90 and 6.95, corresponding to H-2 and H-4, respectively (average enrichment = 1.4%).

We have further resolved the origins of the various hydrogens and oxygens of **5** (Scheme 1). To determine the origins of the oxygens, particularly those at C-5 and C-6, separate fermentations were fed sodium [1-^{13}C,$^{18}O_2$]acetate (**6c**) or grown under an atmosphere of $^{18}O_2$. The former yielded **5d**, and the latter yielded **5e**. Isotope-shifted ^{13}C resonances revealed that **5d** was labelled only at C-1 and C-8. This was in contrast to labelling of **2** from

(12) Guignant, A.; Barreto, M. M. *Tetrahedron Lett.* **1987**, *28*, 3107–3110.

a similar experiment, in which labelling was also observed at C-6 and C-7.[6] The lack of label at C-6 was consistent with the incorporation of **4a**, while the lack of label at C-7 could be explained by exchange with the aqueous fermentation medium.[13] An isotope-shifted natural abundance resonance for C-5 was observed in the spectrum of **5e**, revealing that the oxygen at this position had come from molecular oxygen. The lack of label of C-12 could, once again, be attributed to exchange. Lastly, we determined the fates of the acetate hydrogens by feeding sodium [1-^{13}C,2-$^{2}H_3$]acetate (**6d**), which yielded **5f**. Isotope-induced shifts of the ^{13}C NMR resonances for C-3, -10, and -11a revealed the retention of two deuteriums at C-13 and retention of deuterium at H-9 and H-11. Perhaps significantly, no deuterium had been retained at H-4.

Discussion

The results reported here provide an intimate view of polyketide assembly in benz[*a*]anthraquinone biosynthesis and reveal the nature of the later tailoring events that lead to the most novel part of the structure: the K-region *trans*-diol of PD 116740. The traditional view of deoxygenations in aromatic polyketide biosynthesis has been that they occur prior to cyclizations and aromatizations. In the case of the 6-methylsalicylic acid synthase of *Penicillium patulum*, reduction and dehydration are part of the processive development of the acyclic polyketide intermediate.[14] However, a postaromatic deoxygenation has recently been demonstrated in the conversion of emodin to chrysophanol.[15]

In the case of benz[*a*]anthraquinones, prearomatic deoxygenation at C-10 during formation of the polyketide chain has been assumed, consistent with the ketoreductase and dehydrase genes associated with anthracycline, tetracycline, and benzoisochromanquinone pathways: that is, they are part of the polyketide synthase (PKS).[16] Incorporation of **4** into **5** now indicates deoxygenation at C-6 is also prearomatic, although it remains to be determined whether this is due to a gene integral to the PKS cluster (e.g. acetylCoA to **10** or **11**) or a gene for a subsequent transformation, such as reduction after the formation of a bicyclic intermediate (e.g. **12** to **11**). A partially cyclized intermediate has been proposed in the actinorhodin pathway to rationalize new metabolites produced by blocked mutants.[17] The lack of deuterium enrichment at H-4 of **5f** may also be indicative of partially cyclized intermediates in the biosynthesis of **5**. Tricyclic intermediates have been demonstrated in the biosyntheses of anthracycline antibiotics[18,19] and of tetracenomycin.[20] An analogous bicyclic intermediate has been postulated in actinorhodin biosynthesis.[21] Thus, a tricyclic intermediate **13** in the biosynthesis of benz[*a*]anthraquinones might leave sufficient time for even more extensive exchange adjacent to the residual ketone than is normally encountered at all methylene sites in polyketide and fatty acid biosyntheses.

The conversion of tetrangulol to **5** requires a minimum of four steps in an as yet undefined order: *O*-methylation, arylmethyloxygenation, and a sequence of epoxidation and hydrolysis to generate the *trans*-diol in the K-region of the angular structure. 8-*O*-Methyltetrangulol has been reported as antibiotic X-14881E,[22] and TAN-1085[23] may be a glycoside of 8-desmethyl-**5**, although the diol stereochemistry of this latter antibiotic was not indicated. One example of a benz[*a*]anthraquinone K-region epoxide, elmycin B, has been reported.[2] Aromatic *trans*-diols are rare amongst microbial metabolites[24] (*Pseudomonas* produce dihydrobenzene *cis*-diols by a dioxygenase reaction) but are also found in the benz[*a*]naphthacenequinone antibiotics (e.g. benanomicins[25] and pradimicins[26]), which are homologs of benz[*a*]anthraquinones. Biosynthetically, this latter group is derived from the regular folding of a twenty-six carbon polyketide intermediate,[27,28] but the origins of the oxygens have not yet been identified.

The K-region epoxidation/hydrolysis sequence is an important mammalian metabolism of procarcinogenic polycyclic aromatic hydrocarbons.[29] The epoxide is generally not observed directly and is converted to the diol by an apparently proximal epoxide hydrolase.[30] *In vitro* studies have revealed that the microsomal epoxide hydrolase is by far the most active enzyme with aromatic epoxides.[31,32] The identification of further intermediates in the biosynthesis of PD 116740 and of the enzymology generating the *trans*-diol will be reported in due course.

Experimental Section

Materials and Methods. Reactions were carried out under an Ar atmosphere and anhydrous conditions. THF was distilled from Na/benzophenone ketyl, CH_2Cl_2 from CaH, and MeOH from 4-Å molecular sieves. NH_3 and *N*-methylmorpholine were distilled from Na, and TFA from P_2O_5. Acetylene was purified by bubbling through concentrated H_2SO_4 and then passing over KOH. Flash chromatography was performed using silica gel 60 (particle size 0.040–0.063 mm).

Standard Culture Conditions. *S.* WP 4669 was maintained at 5 °C on agar slants composed of 1.0% cornstarch, 0.2% NZ Amine A, 0.1% Difco beef extract, 0.1% Difco yeast extract, 0.002% $CoCl_2 \cdot 6H_2O$, and 2.0% Bacto agar. Seed cultures were prepared by inoculating 70 mL of medium containing 1.0% glucose, 0.3% NaCl, 0.3% $CaCO_3$, 0.5% soybean meal, and 0.5% glycerol with growth from an agar slant. The cultures, contained in 250-mL Erlenmeyer flasks, were incubated at 28 °C and 250 rpm for 48 h. Production broths (150 mL in 1-L Erlenmeyer flasks), consisting of the same medium, were inoculated 1% v/v with vegetative inoculum from the seed cultures. The production cultures were incubated for 72 h. For precursor feedings, aqueous solutions of the labelled acetates were added in thirds in a sterile manner through Millipore filters (0.2 μm) at 20, 30, and 40 h after inoculation. For the deuteriated **2** and **4**, DMSO solutions were fed in the same manner. Six flasks were used for each feeding experiment.

Isolation. The broth (ca. 900 mL) was filtered through cheese cloth and then passed through a column of Diaion HP-20 resin (100 mL, prepared in H_2O), and the column was sequentially eluted with aqueous 20% MeOH (100 mL), aqueous 50% MeOH (300 mL), and MeOH (100 mL). The material eluted at 50% MeOH was concentrated to dryness, and the residue was dissolved in a small volume of MeOH and applied to a column of silica gel (2.5 × 30 cm^2) prepared in 10% MeOH/$CHCl_3$. Elution with the same solvent yielded a red solution of **5**, which was concentrated to dryness, and the residue was recrystallized from acetone/hexane.

(13) Erickson, W. R.; Gould, S. J. *J. Am. Chem. Soc.* **1985**, *107*, 5831–5832.
(14) Dimroth, P.; Water, H.; Lynen, F. *Eur. J. Biochem.* **1970**, *13*, 98–110.
(15) Anderson, J. A.; Lin, B.-K.; Williams, H. J.; Scott, A. I. *J. Am. Chem. Soc.* **1988**, *110*, 1623–1624.
(16) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37–66.
(17) Zhang, H. I.; He, X.-g.; Adefarati, A.; Galucci, J.; Cole, S. P.; Beale, J. M.; Keller, P. J.; Chang, C.-j.; Floss, H. G. *J. Org. Chem.* **1990**, *55*, 1682–1684.
(18) Wagner, C.; Eckardt, K.; Schumann, G.; Ihn, W.; Tresselt, D. *J. Antibiot.* **1984**, *37*, 691–692.
(19) Schumann, G.; Stengel, C.; Eckardt, K.; Ihn, W. *J. Basic Microbiol.* **1986**, *26*, 249–255.
(20) Shen, B.; Hutchinson, C. R. *Biochemistry* **1993**, *32*, 6656–6663.
(21) Fernandez-Moreno, M. A.; Martinez, E.; Boto, L.; Hopwood, D. A.; Malpartida, F. *J. Biol. Chem.* **1992**, *267*, 19278–19290.
(22) Maehr, H.; Liu, C.-m.; Liu, M.; Perrotta, A.; Smallheer, J. M.; Williams, T. H.; Blount, J. F. *J. Antibiot.* **1982**, *35*, 1627–1631.
(23) Kanamaru, T.; Nozaki, Y.; Muroi, M. Jpn. Patent JP 02,289,532, 1990; *Chem. Abstr.* **1991**, *115*, 47759n.
(24) Jerina, D. M.; Selander, H.; Yagi, H.; Wells, M. C.; Davey, J. F.; Mahadevan, V.; Gibson, D. T. *J. Am. Chem. Soc.* **1976**, *98*, 5988–5996.
(25) Gomi, S.; Sezaki, M.; Kondo, S.; Hara, T.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1988**, *XLI*, 1019–1028.
(26) Tsunakawa, M.; Nishio, M.; Ohkuma, H.; Tsuno, T.; Konishi, M.; Naito, T.; Oki, T.; Kawaguchi, H. *J. Org. Chem.* **1989**, *54*, 2532–2536.
(27) Kakushima, M.; Sawada, Y.; Nishio, M.; Tsuno, T.; Oki, T. *J. Org. Chem.* **1989**, *54*, 2536–2539.
(28) Gomi, S.; Sezaki, M.; Hamada, M.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1989**, *42*, 1145–1150.
(29) Sims, P.; Grover, P. L. In *Advances in Cancer Research*; Klein, G., Weinhouse, S., Eds.; Academic Press: New York, 1974; Vol. 20, pp 165–274.
(30) Daly, J. W.; Jerina, D. M.; Witkop, B. *Experientia* **1972**, *28*, 1129–1264.
(31) Porter, T. D.; Beck, T. W.; Kasper, C. B. *Arch. Biochem. Biophys.* **1986**, *248*, 121–129.
(32) Lu, A. Y. H.; Miwa, G. T. *Annu. Rev. Pharmacol. Toxicol.* **1980**, *20*, 513–531.

Incorporation of Sodium [1-^{13}C,2-^{14}C]Acetate. Sodium [1-^{13}C]acetate (480 mg) and sodium [2-^{14}C]acetate (17.66 μCi) were dissolved in deionized H_2O (36 mL) and fed to six production broths as described above. Workup yielded 13.1 mg of **5a** (0.48 μCi/mmol) after repeated recrystallizations.

Incorporation of Sodium [1,2-$^{13}C_2$,2-^{14}C]Acetate. Sodium [1,2-$^{13}C_2$]-acetate (400 mg) and sodium [2-^{14}C]acetate (17.79 μCi) were fed in the same manner, and 16.4 mg of **5b** (0.56 μCi/mmol) was obtained.

Incorporation of Sodium [1-^{13}C,1-$^{18}O_2$,2-^{14}C]Acetate. Sodium [1-^{13}C,1-$^{18}O_2$]acetate (400 mg) and sodium [2-^{14}C]acetate (26.80 μCi) were fed in the same manner, and 8.1 mg of **5d** (0.69 μCi/mmol) was obtained.

Incorporation of Sodium [1-^{13}C,2-$^{2}H_3$,2-^{14}C]Acetate. Sodium [1-^{13}C,2-$^{2}H_3$]acetate (600 mg) and sodium [2-^{14}C]acetate (19.73 μCi) were fed in the same manner, and 9.2 mg of **5f** (0.57 μCi/mmol) was obtained.

Incubation of S. WP 4669 in the Presence of $^{18}O_2$. Seventy milliliters of a 48-h seed culture, 900 mL of sterile production medium, and 1 mL of Antifoam A (Sigma Chemical Co.) were combined in the sterile fermentor apparatus described by Vederas.[33] The buret was initially charged with $^{16}O_2$, and during the first 38 h, 440 mL was consumed. The buret was then charged with 50% $^{18}O_2$ and replenished as needed; 3100 mL was consumed during the next 74 h. $^{16}O_2$ was used for an additional 9 h, and workup gave 22 mg of **5e**.

Incorporation of [2,4-$^{2}H_2$]Tetrangulol. On two separate occassions, [2,4-$^{2}H_2$]tetrangulol (20 mg) in DMSO (2 mL) was fed as described above. After 72 h, workup yielded ca. 15 mg of **5c** each time: ^{2}H NMR (MeOH) δ 6.85–7.05. Quantitation using the methanol resonance indicated enrichments at each site of ca. 1.4% (24 402 scans) in one experiment and 2.9% (23 722 scans) in the other.

Synthesis of Tetrangulol (4a). A. 3-Ethoxy-5-methylcyclohex-2-en-1-one.[34] *p*-TsOH (0.654 g, 3.44 mmol) and absolute EtOH (22 mL) were added to 5-methyl-1,3-cyclohexanedione (15.0 g, 119 mmol) in benzene (78 mL) in a flask fitted with a Soxlet extractor charged with Drierite (36 g). The mixture was heated at reflux for 28 h and allowed to cool, and 2,6-di-*tert*-butyl-4-methylphenol (15 mg) added. The mixture was washed sequentially with 10% NaOH in saturated brine (4 × 30 mL), H_2O (3 × 15 mL), and saturated brine (1 × 15 mL). After drying and filtering, concentration gave a faintly yellow oil, which was distilled to give 16.4 g (89%) of 3-ethoxy-5-methylcyclohex-2-en-1-one as a clear oil: bp 64–76 °C/7 μm; UV (EtOH) λ_{max} 248 nm (ε 16 000); IR (KBr) 1653.6, 1602.4 cm^{-1}; ^{1}H HMR ($CDCl_3$) δ 1.01 (d, 3H, *J* = 6.1 Hz), 1.27 (t, 3H, *J* = 7.0 Hz), 1.98 (m, 2H), 2.14 (m, 1H), 2.34 (m, 2H), 3.83 (m, 2H), 5.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 199.7, 177.2, 102.2, 64.1, 45.0, 37.1, 28.7, 20.8, 14.0; EIMS *m/z* (relative intensity) 154.1 (M^+, 56%), 112.0 (100%); HREIMS *m/z* calcd for $C_9H_{14}O_2$ 154.0994 (M^+), found 154.0994.

B. 3-Ethynyl-5-methylcyclohex-2-en-1-one.[35,36] NH_3 (300 mL) was distilled onto Li wire (ca. 0.5 mol). The resulting blue solution was agitated with a Hirshberg stirrer under an acetylene atmosphere at −78 °C until the blue color disappeared. Evaporation of the NH_3 at room temperature over 48 h provided a white precipitate which was suspended in THF. 3-Ethoxy-5-methylcyclohex-2-en-1-one (13.1 g, 84.7 mmol) in THF (100 mL) was added over 2 h while maintaining an acetylene atmosphere and agitating by sonication. In 3 h total reaction time the bath temperature had risen to 45 °C, and the reaction appeared complete by TLC (20% EtOAc in hexane). The mixture was quenched by addition of H_2SO_4 (200 mL, 2.5 N, 1 equiv/Li), and the biphasic mixture was extracted with CH_2Cl_2 (5 × 100 mL). After the combined organic layers were dried over a mixture of Na_2SO_4/Na_2CO_3 (10:1), 2,6-di-*tert*-butyl-4-methylphenol (50 mg) was added and the mixture was concentrated to provide a brown oil which was distilled to give 5.67 g (50%) of 3-ethynyl-5-methylcyclohex-2-en-1-one: bp 61 °C/5 μm; UV (EtOH) λ_{max} 250 nm (ε 8500); IR (NaCl) 2094, 1662, 1589 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.28 (s, 2H), 3.56 (s, 1H), 2.52 (m, 1H), 2.19 (m, 3H), 1.10 (d, 3H, *J* = 3.9 Hz); ^{13}C NMR ($CDCl_3$) δ 199.0, 141.5, 133.6, 87.1, 82.5, 45.4, 38.2, 30.0, 20.9; EIMS *m/z* (relative intensity) 134.1 (M^+, 31%), 92.1 (100%), 77.1 (9%); HREIMS *m/z* calcd for $C_9H_{10}O$ 134.0732 (M^+), found 134.0732.

C. (*E,E*)-3-(2-Methoxyethenyl)-5-methylcyclohexenone.[37] *N*-methylmorpholine (3.13 mL, 2.88 g, 28.5 mmol), 2,6-di-*tert*-butyl-4-methylphenol (50 mg), and MeOH (46.2 mL, 36.5 g, 1.14 mol) were added sequentially to 3-ethynyl-5-methylcyclohex-2-en-1-one (3.83 g, 28.5 mmol) in dry CH_2Cl_2 (285 mL). The mixture was protected from light and stirred at 22 °C under Ar for 24 h, at which time it had turned dark red. After the mixture was washed with H_2O (2 × 100 mL) and dried, the mixture was loaded without concentration onto a silica gel column (4 × 10 cm) packed in CH_2Cl_2. Elution with 5% EtOAc/CH_2Cl_2 followed by concentration afforded 4.07 g (86%) of (*E,E*)-3-(2-methoxyethenyl)-5-methylcyclohexenone as a colorless oil: IR (KBr) 1617.4 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 3H, *J* = 6.4 Hz), 2.06 (m, 2H), 2.20 (m, 1H), 2.46–2.56 (m, 2H), 3.71 (s, 3H), 5.64 (d, 1H, *J* = 12.9 Hz), 5.84 (d, 1H, *J* = 1.6 Hz), 7.04 (d, 1H, *J* = 12.9 Hz); ^{13}C NMR ($CDCl_3$) δ 199.7, 156.3, 154.3, 123.8, 106.9, 57.1, 45.7, 33.6, 29.8, 21.3; EIMS/*m/z* (relative intensity) 166.1 (M^+, 14%), 151.1 (3%), 124.0 (24%), 112.1 (16%), 78.1 (100%), 67.0 (17%); HREIMS *m/z* calcd for $C_{10}H_{14}O_2$ 166.0994 (M^+), found 166.0994.

D. Ochromycinone (8).[12] A solution of (*E,E*)-3-(2-methoxyethenyl)-5-methylcyclohexenone (4.07 g, 24.5 mmol) and juglone (6.30 g, 36.2 mmol, recrystallized from hexane) in CH_2Cl_2 (150 mL) was treated with a suspension of boron triacetate[38] (11.0 g, 58.5 mmol) in CH_2Cl_2 (60 mL) over 6 h. After an additional 20 h of stirring, MeOH (17.3 mL, 427 mmol, 2.4 equiv/acetate) was added and the mixture was filtered through Celite and loaded onto a silica gel column (5 × 7 cm) packed in CH_2Cl_2. Elution with CH_2Cl_2/acetone (1:1) and concentration gave a black paste which was further chromatographed on silica gel (5 × 20 cm, eluted with CH_2Cl_2) to give 2.45 g of **8** after recrystallization (EtOAc/hexane). The mother liquor and mixed fractions were rechromatographed to afford an additional 0.960 g after recrystallization, for a total of 3.41 g (46%) of **8** as yellow plates: mp 173.4–173.8 °C (lit. 152–153 °C,[39] 168–169 °C[40]); IR (KBr) 1703.4, 1635.1 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 12.29 (s, 1H), 8.28 (d, 2H, *J* = 8.0 Hz), 7.66–7.69 (m, 2H), 7.55 (d, 1H, *J* = 8.2 Hz), 7.25–7.28 (m, 1H), 2.97–3.07 (m, 2H), 2.53–2.73 (m, 2H), 2.45–2.49 (m, 1H), 1.21 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 199.2, 187.5, 183.0, 162.0, 150.4, 137.0, 136.6, 135.9, 135.0, 133.4, 133.0, 128.9, 123.6, 119.6, 115.4, 47.5, 38.4, 30.8, 21.5; EIMS *m/z* (relative intensity) 306.3 (M^+, 64%), 264.2 (100%); HREIMS *m/z* calcd for $C_{19}H_{14}O_4$ 306.0892 (M^+), found 306.0892.

E. 2-Bromoochromycinone (9).[41,42] A mixture of $CHCl_3$ and EtOAc (1:1, 150 mL, dried over $CaSO_4$) was added to **8** (1.36 g, 4.45 mmol) and $CuBr_2$ (1.59 g, 7.12 mmol, dried at room temperature under vacuum). The mixture was heated at reflux under Ar until the black color of the cupric bromide turned to a light gray (4 h) and was then filtered and concentrated. Chromatography on silica gel (5 × 16 cm) eluted with 20% toluene in CH_2Cl_2 first yielded 1.29 g of **9** (86%, based on unrecovered starting material) as a yellow solid (an inseparable 5:1 mixture of *trans* and *cis* diastereomers), followed by recovered **8** (0.174 g): mp 210.0–211.4 °C (dec to tetrangulol); IR 1710.7, 1676.0, 1635.5, 1591.7 cm^{-1}; (major isomer) ^{1}H NMR ($CDCl_3$) δ 12.25 (s, 1H), 8.29 (d, 1H, *J* = 8.1 Hz), 7.69–7.77 (m, 2H), 7.50 (d, 1H, *J* = 8.2 Hz), 7.31 (d, 1H, *J* = 8.0 Hz), 4.58 (d, 1H, *J* = 2.4 Hz), 2.98 (dd, 2H, *J* = 8.0, 2.0 Hz), 2.57 (m, 1H), 1.27 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 192.1, 187.2, 182.1, 161.9, 148.4, 137.1, 134.8, 133.9, 133.6, 133.0, 129.5, 129.3, 123.6, 119.6, 115.2, 59.0, 35.4, 34.4, 18.8; EIMS *m/z* (relative intensity) 386.1 ([M + 2]$^+$, 12%), 384.1 (M^+, 11%), 305.2 (75%), 264.2 (100%); HREIMS *m/z* calcd for $C_{19}H_{13}{}^{79}BrO_4$ 383.9998 (M^+), found 383.9997.

F. Tetrangulol (4).[10,43] A solution of **9** (1.23 g, 3.19 mmol) in DMF (40 mL) was added to a hot (135 °C), stirred suspension of LiBr (5.54 g, 63.8 mmol) and Li_2CO_3 (4.72 g, 63.9 mmol) in DMF (140 mL) over 0.25 h.[44,45] The reaction was cooled to room temperature, filtered through Celite, concentrated to ca. 50 mL, and diluted with toluene (150 mL). The mixture was filtered through silica gel (5 × 5 cm) and the column

(33) Moore, R. N.; Bigam, G.; Chan, J. K.; Hogg, A. M.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 3694–3701.
(34) Gannon, W. F.; House, H. O. 3-Ethoxy-2-cyclohexenone. In *Organic Syntheses*; Baumgarten, H. E., Ed.; John Wiley and Sons: New York, 1973; Collect. Vol. V, pp 539–541.
(35) Hoffman, J. W.; Arapakos, P. J. *J. Org. Chem.* **1965**, *30*, 1604–1607.
(36) Raphael, R. A. *Acetylenic Compounds in Organic Synthesis*; Academic: New York, 1955; pp 193–194.
(37) Shostakovskii, M. F.; Trofimov, B. A.; Atavin, A. S.; Lavrov, V. I. *Russ. Chem. Rev.* **1968**, *37*, 907–918.
(38) Cook, H. G.; Ilett, J. D.; Saunders, B. C.; Stacey, G. J. *J. Chem. Soc.* **1950**, 3125–3128.
(39) Thomson, R. H. *Naturally Occurring Quinones*, 2nd ed.; Academic: New York, 1971; p 648.
(40) Katsuura, K.; Snieckus, V. *Tetrahedron Lett.* **1985**, *26*, 9–12.
(41) Bauer, D. P.; Macomber, R. S. *J. Org. Chem.* **1975**, *40*, 1990–1992.
(42) King, L. C.; Ostrum, G. K. *J. Org. Chem.* **1964**, *29*, 3459–3461.
(43) Brown, P. M.; Thomson, R. H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 997–1000.
(44) Demuth, M. R.; Garret, P. E.; White, J. D. *J. Am. Chem. Soc.* **1976**, *98*, 634–635.
(45) Stotter, P. L.; Hill, K. A. *J. Org. Chem.* **1973**, *38*, 2576–2578.

washed with additional toluene. Concentration of the combined fractions gave 1.00 g (100%) of **4** as purple/brown needles: mp 198.2–200.8 °C (lit. 201–203 °C); IR 3428.2, 1631.4, 1617.7 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H), 7.14 (s, 1H), 7.26 (s, 1H), 7.33 (dd, 1H, J = 8.4, 0.9 Hz), 7.69 (t, 1H, J = 8.0 Hz), 7.85 (dd, 1H, J = 6.4, 0.8 Hz), 8.12 (d, 1H, J = 8.7 Hz), 8.30 (d, 1H, J = 8.5 Hz), 11.28 (s, 1H), 12.26 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 189.6, 187.8, 161.6, 155.2, 142.0, 139.1, 137.7,[46] 136.9, 134.7, 132.3, 124.7, 121.9, 121.2,[46] 120.2, 120.0, 114.5, 21.3; EIMS m/z (relative intensity) 304.3 (M^+, 100%); HREIMS m/z calcd for $C_{19}H_{12}O_4$ (M^+) 304.0736, found 304.0736.

G. [2,4-$^2H_2$]Tetrangulol (4a). TFA (12.4 g, 59.2 mmol) and D_2O (1.34 g, 66.7 mmol) were stirred together at 0 °C for 1 h to give TFA-*d*. **4** (50.3 mg, 0.165 mmol) and TFA-*d* (2.00 mL, 25.9 mmol, 160 equiv) were mixed in a heavy-walled tube, and the suspension was frozen (dry ice–acetone), evacuated, and sealed. After being heating in an oil bath (110–120 °C) for 1.5 days, the mixture was cooled and concentrated *in vacuo*, and the remaining brown solid was suspended in $CHCl_3$ and filtered through a silica gel plug (1 × 2.5 cm). Concentration gave 49.4 mg (98%) of pure **4a** as purple needles. ^2H NMR and EIMS showed 97% ^2H per exchanged position: mp 201.6–202.8 °C; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H), 7.33 (dd, 1H, J = 8.4, 0.9 Hz), 7.69 (t, 1H, J = 8.0 Hz), 7.85 (dd, 1H, J = 6.4, 0.8 Hz), 8.12 (d, 1H, J = 8.7 Hz), 8.28 (d, 1H, J = 8.5 Hz), 11.26 (s, 1H), 12.23 (s, 1H); ^2H NMR (dioxane) δ 7.12 (s, 1 ^2H), 7.31 (s, 1 ^2H); ^{13}C NMR ($CDCl_3$) δ 189.5, 187.7, 161.6, 155.2, 141.8, 139.0, 137.5, 136.8, 134.7, 132.2, 124.7, 121.8, 121.2, 121.1 (t, J = 26.6 Hz), 120.1 (t, J = 23.5 Hz), 119.9, 114.5, 21.1; EIMS m/z (relative intensity) 306.3 ($[M + 2]^+$, 100%), 305.3 ($[M + 1]^+$, 6%); HREIMS m/z calcd for $C_{19}H_{10}{}^2H_2O_4$ (M^+) 306.0861, found 306.0861.

(46) This resonance is a single line at 75 MHz but resolves into two lines at 100 MHz.

Acknowledgment. This work was supported by U.S. Public Health Service Grant GM 31715 to S.J.G. Dr. J. C. French is thanked for arranging receipt of a slant of *S.* WP 4669 and an authentic sample of PD 116740 from the Warner-Lambert Company. The Bruker AM 400 NMR spectrometer was purchased in part through grants from the National Science Foundation (CHE-8216190) and from the M. J. Murdock Charitable Trust to Oregon State University, and the Bruker AC 300 spectrometer was purchased in part through grants from the Public Health Service Division of Research Resources (RR04039-01) and the National Science Foundation (CHE-8712343) to Oregon State University.