hypochlorite in a ratio of 3:1 was irradiated at 0 °C until the yellow color had disappeared and then for an additional 0.5 h. Preparative GC was used to collect the three new products. The major product (75%) was identified as 2-chloro-1,2-epoxypropane (2). $^1$H NMR (300 MHz, $CDCl_3$): δ 3.10 (1 H, d, $J$ = 4.9 Hz), 2.83 (1 H, d, $J$ = 4.9 Hz), 1.89 (3 H, s, $CH_3$). The other products (15 and 10%) were identified as *trans*- and *cis*-1-chloro-1,2-epoxypropane (3 and 4), respectively. $^1$H NMR (3) (300 MHz, $CDCl_3$): δ 4.82 (1 H, d, $J$ = 1.0 Hz), 3.22 (1 H, dq, $J$ = 1.0 and 5.2 Hz) 1.36 (3 H, d, $J$ = 5.2 Hz). $^1$H NMR (4) (300 MHz, $CDCl_3$): δ 5.16 (1 H, d, $J$ = 2.8 Hz), 3.18 (1 H, dq, $J$ = 2.8 and 5.4 Hz), 1.49 (3 H, d, $J$ = 5.4 Hz).

**Preparation of *erythro*- and *threo*-3-Chloro-1,2-epoxybutane.** Epoxidation of 3-chloro-1-butene with *m*-CPBA in refluxing $CH_2Cl_2$ gave a 1:1 mixture of the erythro and threo compounds 5 and 6. Isolation of each isomer was accomplished by preparative GC. $^1$H NMR (5) (300 MHz, $CDCl_3$): δ 3.64 (1 H, p, $J$ = 6.7 Hz), 3.08 (1 H, m), 2.88 (1 H, t, $J$ = 4.2 Hz), 2.69 (1 H, dd, $J$ = 4.7 and 2.5 Hz), 1.61 (3 H, d, $J$ = 6.6 Hz). $^{13}$C NMR (5) (75.5 MHz, $CDCl_3$): δ 56.8, 55.5, 47.2, 21.8. $^1$H NMR (6) (300 MHz, $CDCl_3$): δ 3.80 (1 H, p, $J$ = 6.7 Hz), 3.15 (1 H, m), 2.89 (1 H, dd, $J$ = 4.7 and 4.0 Hz), 2.72 (1 H, dd, $J$ = 4.8 and 2.5 Hz), 1.55 (3 H, d, $J$ = 6.7 Hz). $^{13}$C NMR (6) (75.5 MHz, $CDCl_3$): δ 57.6, 55.7, 46.4, 20.6.

**Preparation of *trans*- and *cis*-2-Chloro-7-oxabicyclo[4.1.0]heptane.** Epoxidation of 3-chlorocyclohexene with *m*-CPBA in refluxing $CH_2Cl_2$ gave a 9:1 mixture of compounds 7 and 8. Separation and isolation of each isomer was accomplished by preparative GC. Treatment of each isomer with an equivalent of concd HCl gave a single isomer of 1,3-dichlorocyclohexan-2-ol (see text). $^1$H NMR (7) (300 MHz, $CDCl_3$): δ 4.35 (1 H, t, $J$ = 4.8 Hz), 3.29 (1 H, d, $J$ = 4.2 Hz), 3.25 (1 H, t, $J$ = 3.4 Hz), 1.96 (3 H, m), 1.60 (2 H, m), 1.32 (1 H, m). $^{13}$C NMR (7) (75.5 MHz, $CDCl_3$): δ 55.0, 54.9, 52.2, 28.8, 23.1, 15.1. HRMS: calcd for $^{12}C_6{}^1H_9{}^{16}O_1{}^{35}Cl_1$ 132.03419, found 132.03419, $^1$H NMR (8) (300 MHz, $CDCl_3$): δ 4.27 (1 H, ddd, $J$ = 10.0, 5.3, and 1.9 Hz), 3.32 (2 H, m), 1.70 (5 H, m), 1.27 (1 H, m). $^{13}$C NMR (8) (75.5 MHz, $CDCl_3$): δ 57.4, 56.5, 55.3, 29.4, 22.3, 20.7. HRMS: calcd for $^{12}C_6{}^1H_9{}^{16}O_1{}^{35}Cl_1$ 132.03419, found 132.03419.

**Kinetics.** All kinetic studies were run in replicate on pairs of substrates. The two substrates, along with $Ph_3SnH$, an internal standard, and a solvent (benzene or cyclohexane) were mixed in an approximate ratio of 1:1:1:0.5:10. Aliquots were sealed in Pyrex ampules, under a reduced pressure of $N_2$, after three freeze–thaw cycles. In each case one of the ampules was reserved for analysis of starting material. The reactions were run in a temperature-controlled oil bath at 70 ± 0.5 °C. Reaction times were varied in order to achieve 15–90% reaction of each substrate. Relative rates were determined by disappearance of starting material, as measured by integration of $^1$H NMR (300 MHz) or capillary GC.

**Registry No.** 1, 106-89-8; 2, 5950-21-0; 3, 21947-76-2; 4, 21947-75-1; 5, 52066-40-7; 6, 52066-41-8; 7, 137940-88-6; 8, 137940-89-7; 1-chloro-2-methoxyethane, 627-42-9; benzyl chloride, 100-44-7; cyclohexyl chloride, 542-18-7; neophyl chloride, 515-40-2; propylene oxide, 75-56-9; 3-chloro-2-butene, 563-52-0; 3-chlorocyclohexene, 2441-97-6; triphenylstannane, 892-20-6; triphenylstannyl, 17272-58-1.

# Isolation and Structure Determination of Pentalenolactones A, B, D, and F

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Four new metabolites related to pentalenolactone have been isolated, pentalenolactone A (17), B (18), D (7), and F (10), and their structures established by a combination of $^1$H and $^{13}$C NMR, $^1$H NOE, and $^1$H COSY spectroscopy, assisted by molecular modeling calculations. The structure and stereochemistry of pentalenolactone D phenacyl ester (21) was established by X-ray crystallography. Each of these metabolites may be important intermediates or shunt metabolites in the biosynthesis of pentalenolactone (16).

The sesquiterpene antibiotic pentalenolactone (16), which has been isolated from a variety of *Streptomyces* species, is a rare example of a cyclic terpenoid produced by a prokaryotic organism (Scheme I). Following the original isolation in 1957,[1] the structure and absolute configuration were eventually assigned in 1970 by a combination of spectroscopic and X-ray crystallographic methods.[2,3] In addition to exhibiting a broad spectrum of activity against a wide variety of organisms, including Gram-positive and Gram-negative bacteria, pentalenolactone has been shown to block glycolysis by selective inhibition of glyceraldehyde-3-phosphate dehydrogenase from both prokaryotic (*Escherichia coli, Bacillus subtilis*) as well as eukaryotic sources (yeast, spinach, rabbit muscle).[4] Pentalenolactone has also been reported to exhibit potent and specific antiviral activity.[5] Studies in our own laboratory have shown that pentalenolactone is a time-dependent, irreversible inactivator of glyceraldehyde-3-phosphate dehydrogenase whose inhibitory action is due to specific reaction with all four active-site cysteines of the tetrameric enzyme.[6] Additional studies with model thiols have suggested that the thiol residue is alkylated by ring opening at C-10 of the epoxy lactone moiety although this has yet to be demonstrated directly for inactivation of the enzyme itself.[6b]

We have demonstrated the sesquiterpenoid biosynthetic origin of pentalenolactone[7] and carried out extensive

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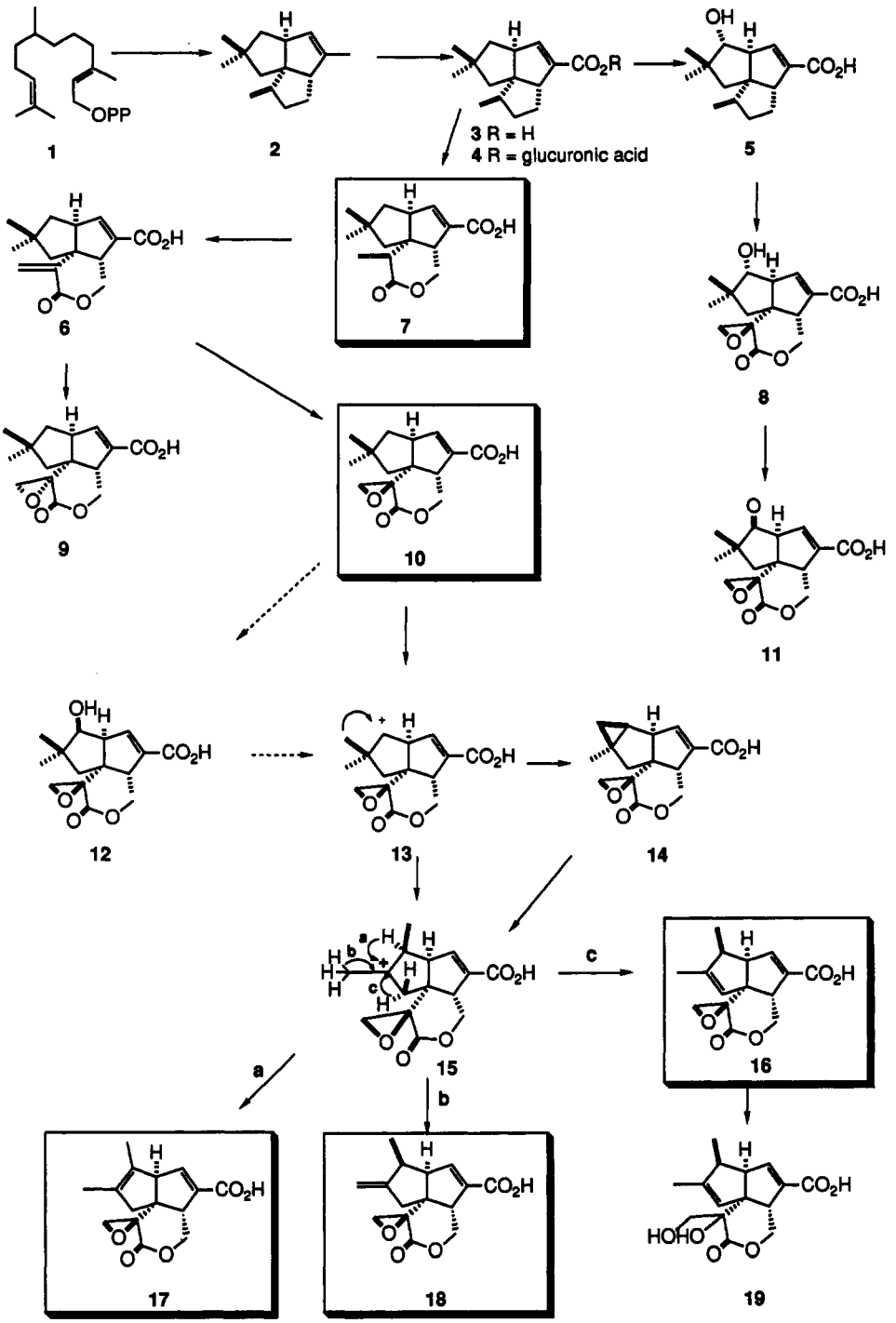
0022-3263/92/1957-0844$03.00/0 

Scheme I




studies of the mechanism and stereochemistry of the enzymatic cyclization of farnesyl diphosphate (1) to pentalenene (2), the parent hydrocarbon of the pentalenolactone family of metabolites.[8] The cyclase itself, pentalenene synthase, a monomer of $M_R$ 42.5 kDa, has recently been purified to homogeneity and partially sequenced.[6b,9] Labeled pentalenene is converted to pentalenolactone by cultures of *Streptomyces* UC5319.[8]

Pentalenolactone has been isolated from several species of *Streptomyces*, including *S. chromofuscus*, *S. griseochromogenes*, *S. baarnensis*, *S. arenae*, *S. roseogriseus*, and *S.* UC5319. Accompanying pentalenolactone are numerous cometabolites representing possible intermediates or shunt metabolites of the biosynthetic conversion of pentalenene to pentalenolactone. For example, Seto et al. have isolated pentalenolactone G (11),[10] pentalenolactone H (8),[11] pentalenolactone O (19),[12] pentalenolactone P (14),[12] and

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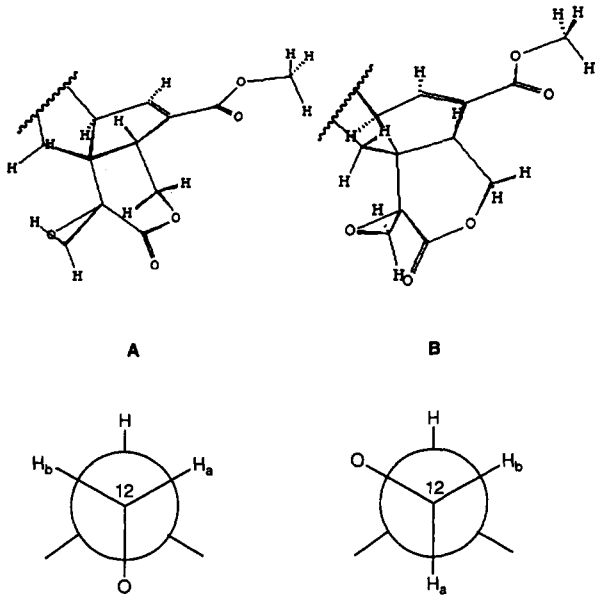
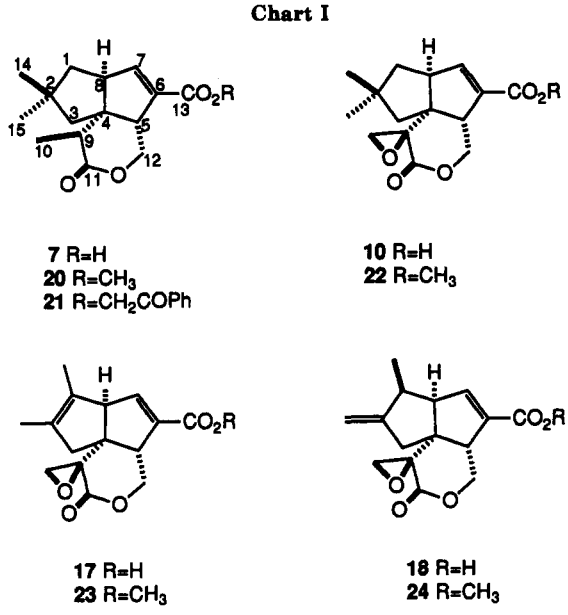



**Figure 1.** Gauche (**A**) and anti (**B**) lactone conformations of pentalenolactone metabolites.

pentalenic acid (**5**)[11] from *S. chromofuscus*, as well as the parent sesquiterpene hydrocarbon, pentalenene (**2**), from *S. griseochromogenes*.[13] In addition, Takahashi et al. have isolated deoxypentalenylglucuron (**4**)[14] from *S. omiyaensis*, *S. albofaciens*, and *S. viridifaciens*.

Our own group has reported the isolation and structure determination of both pentalenolactone E (**6**)[15] and what is now termed *epi*-pentalenolactone F (**9**),[16] obtained from the fermentation broth of *S.* UC5319. The structure of the latter metabolite was originally assigned as **10** on the basis of $^1$H NMR and $^{13}$C NMR spectroscopy,[16a] the epoxide stereochemistry being inferred by analogy to the known configuration of pentalenolactone (**16**) and that of the cometabolites pentalenolactones G (**11**) and H (**8**), as well as on the basis of chemical arguments.[17] The epoxide configurational assignments were subsequently cast into doubt by further NMR studies carried out by Matsumoto et al.[18] The Hokkaido group pointed out that, based on the chemical shifts for H-12$_a$ and H-12$_b$ as well as the observed $J_{H\text{-}5,H\text{-}12}$ coupling constants, the methyl esters of synthetic 9-*epi*-pentalenolactone H and what was then termed pentalenolactone F, but now *epi*-pentalenolactone F, appeared to exist in lactone conformation **A** (Figure 1) in which the C(5)–H(5) bond is gauche to both the C(12)–H(12$_a$) and C(12)–H(12$_b$) bonds, whereas the methyl esters of pentalenolactones G and H as well as the synthetic epoxide epimer **22**, now called pentalenolactone F methyl ester, existed in a second conformation (**B**), in which the C(5)–H(5) and C(12)–H(12$_a$) bonds have an anti relationship. When X-ray crystallographic analysis by Seto et al. reconfirmed the correctness of the epoxide stereochemical assignment for pentalenolactone G methyl ester,[10a] we carried out our own X-ray crystallographic study, leading to the now accepted revised structure **9** for naturally occurring *epi*-pentalenolactone F.[16b] Pentalenolactone and its structurally related cometabolites have been the targets of numerous synthetic investigations.

**Chart I**




As reported below, we have now isolated the methyl ester of yet another metabolite, termed pentalenolactone F (**10**), having the 9*R* epoxide configuration corresponding to the majority of naturally occurring pentalenolactones. In addition, detailed examination of the culture broths of *S.* UC5319 has led to the isolation of three new biogenetically related metabolites, pentalenolactones A (**17**), B (**18**), and D (**7**), as described below (Scheme I and Chart I).

## Results

Treatment of the crude chloroform extracts of the acidified broth from a large-scale fermentation culture of *S.* UC5319 with ethereal diazomethane gave a mixture of methyl esters which was subjected to extensive purification by a combination of silica gel flash chromatography, medium and high pressure liquid chromatography, and TLC. The purified individual components were then subjected to extensive NMR analysis. In one case, the structure and stereochemistry of pentalenolactone D was further confirmed by X-ray crystallographic analysis of the corresponding phenacyl ester **21**.

**Pentalenolactone D.** The 400-MHz $^1$H NMR spectrum of pentalenolactone D methyl ester (**20**) showed a triplet at $\delta$ 6.83, characteristic of the conjugated olefinic (H-7) proton present in the majority of the known pentalenene or pentalenolactone metabolites. Another characteristic pair of doublets at $\delta$ 1.17 ($J$ = 8.9 Hz, H-10, 3 H) and a quartet at $\delta$ 2.71 ($J$ = 6.6 Hz, H-9, 1 H) indicated the coupling of a methyl group (H-10) and a methine proton (H-9). The high resolution mass spectrum of **20** established the elemental composition as $C_{16}H_{22}O_4$.

Pentalenolactone D phencyl ester (**21**) was recrystallized by the vapor diffusion method from pentane–THF (mp 158.5–159 °C).[19] The crystal of pentalenolactone D phenacyl ester grew in the monoclinic space group $P2_1$. The unit cell parameters were determined to be $a$ = 9.267 (3) Å, $b$ = 10.634 Å (3), $c$ = 10.518 Å (3), and $\alpha = \gamma = 90°$, $\beta$ = 93.51 (5)°, by least-squares fitting to the positions of 25 independent reflections in the range 24 ° ≤ 2$\theta$ ≤ 34°. This unit cell contained two asymmetric units of molecular formula $C_{23}H_{26}O_5$ in a volume of 1034.35 (0.43) Å$^3$, which

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Table I. $^1$H NMR Spectral Data ($CDCl_3$) of the Methyl Esters of Pentalenolactones D (20), F (22), A (23), and B (24) and *epi*-Pentalenolactone F (9-Me)

| 20 | | 22 | | 9-Me | | 23 | | 24 | |
|---|---|---|---|---|---|---|---|---|---|
| H | δ (m, J, area) | H | δ (m, J, area) | H | δ (m, J, area) | H | δ (m, J, area) | H | δ (m, J, area) |
| 7 | 6.83 (t, 2.36, 2.32 Hz, 1 H) | 7 | 6.84 (t, 2.3, 2.4 Hz, 1 H) | 7 | 6.84 (bt, 1 H) | 7 | 6.94 (t, 2.3, 2 Hz, 1 H) | 7 | 6.69 (t, 2.2, 2.3 Hz, 1 H) |
| 12α | 4.81 (dd, 11.7, 7.0 Hz, 1 H) | 12β | 4.80 (dd, 6, 11.5 Hz, 1 H) | 12a | 4.77 (dd, 11.5, 2.2 Hz, 1 H) | 12b | 4.75 (dd, 4.8, 11.8 Hz, 1 H) | 15a | 4.87 (s, 1 H) |
| 12β | 3.97 (dd, 11.7, 11.7 Hz, 1 H) | 12α | 4.18 (dd, 9, 11.5 Hz, 1 H) | 12b | 4.44 (dd, 11.5, 2.2 Hz, 1 H) | 12a | 4.47 (dd, 4.7, 11.8 Hz, 1 H) | 12b | 4.75 (dd, 5.1, 11.6 Hz, 1 H) |
| 16 | 3.72 (s, 3 H) | 16 | 3.74 (s, 3 H) | 16 | 3.74 (s, 3 H) | 16 | 3.75 (s, 3 H) | 15b | 4.71 (s, 1 H) |
| 5 | 3.36 (m, 1 H) | 5 | 3.55 (m, 1 H) | 5, 8 | 3.44 (m, 2 H) | 8 | 3.5 (bs, 1 H) | 12a | 4.35 (dd, 6.6, 11.6 Hz, 1 H) |
| 8 | 3.12 (m, 1 H) | 8 | 2.98 (m, 1 H) | 10 | 2.97 (dd, 5.2 Hz, 2 H) | 5 | 3.34 (m, 1 H) | 16 | 3.73 (s, 3 H) |
| 9 | 2.71 (q, 6.6 Hz, 1 H) | 10b | 3.04 (d, 4.7 Hz, 1 H) | 1, 3 | 1.7, 1.43 (m, 4 H) | 10a | 3.21 (d, 4.6 Hz, 1 H) | 5 | 3.25 (m, 1 H) |
| 3 | 1.78 and 1.35 (AB q, 13.8 Hz, 2 H) | 10a | 2.92 (d, 4.7 Hz, 1 H) | 14, 15 | 1.0 (s), 0.98 (s) (6 H) | 10b | 2.70 (d, 4.6 Hz, 1 H) | 10a | 3.18 (d, 4.64 Hz, 1 H) |
| 1α | 1.65 (dd, 10.4, 2.84 Hz, 1 H) | 3α | 1.88 (d, 12.5 Hz, 1 H) | | | 3α | 2.46 (d, 16 Hz, 1 H) | 8 | 3.09 (m, 1 H) |
| 1β | 1.53 (dd, 10.4, 1.2 Hz, 1 H) | 3β | 1.69 (d, 12.5 Hz, 1 H) | | | 3β | 2.26 (d, 16 Hz, 1 H) | 10b | 2.98 (d, 4.64 Hz, 1 H) |
| 10 | 1.17 (d, 8.9 Hz, 3 H) | 1β | 1.72 (dd, 9.6, 13 Hz, 1 H) | | | 14 | 1.59 (s, 3 H) | 1 | 2.6 (m, 1 H) |
| 14 | 1.00 (s, 3 H) | 1α | 1.43 (dd, 6.5, 13 Hz, 1 H) | | | 15 | 1.54 (s, 3 H) | 3α | 2.52 (d, 15.16 Hz, 1 H) |
| 15 | 0.99 (s, 3 H) | 14, 15 | 1.03 (s, 6 H) | | | | | 3β | 2.24 (d, 15.16 Hz, 1 H) |
| | | | | | | | | 14 | 1.03 (d, 6.92 Hz, 3 H) |

produced a calculated density of 1.23 g/cm$^3$. A total of 1701 reflections were recorded in the range $3.5° \leq 2\theta \leq 46°$ with a Nicolet R3m/E crystallographic system using the $\theta$:$2\theta$ scan routine and graphite-monochromated Mo K$\alpha$ radiation ($\lambda$ = 0.71073 Å). A total of 1380 unique reflections were observed using the criterion [$F_o \geq 3.0(F_o)$]. After Lorentz and polarization corrections, the structure was solved by the SHELXTL 5.1 programs. All non-hydrogen atoms were refined anisotropically. The approximate locations of all hydrogen atoms were placed in calculated positions and allowed to ride with the atom to which they are attached.[20]

From the X-ray structure, the measured dihedral angles in the lactone ring were H–C(5)–C(12)–$H_\beta$ = 48.2° and H–C(5)–C(12)–$H_\alpha$ = 168.1°. These dihedral angles are consistent with the observed coupling constants $J_{H\text{-}5,H\text{-}12}$ = 7 and 11.4 Hz of pentalenolactone D phenacyl ester (21). Pentalenolactone D (7), which can be derived biogenetically by oxidation of pentalenene is conceivably a direct precursor of pentalenolactone E (6).

**Pentalenolactone F.** Further examination of extracts of *S.* UC5319 led to isolation of a new metabolite, pentalenolactone F (10), the epoxide epimer of the previously discussed *epi*-pentalenolactone F (9). The detailed structure of pentalenolactone F methyl ester (22) was assigned largely on the basis of NMR spectroscopic data. The high resolution mass spectrum of 22 established the elemental composition as $C_{16}H_{20}O_5$. The infrared spectrum exhibited bands characteristic of a lactone (1785 cm$^{-1}$), an ester (1714 cm$^{-1}$), and a double bond (1620 cm$^{-1}$) with no hydroxyl group absorption. The $^1$H, $^{13}$C, and $^1$H COSY NMR spectra of 22 were very similar to those of *epi*-pentalenolactone F methyl ester (9) (Tables I and II).

The $^1$H NMR spectrum of 22 showed a characteristic triplet-like peak ($J$ = 2.3 and 2.4 Hz, 1 H) at δ 6.84 for the H-7 conjugated olefinic proton coupled with H-8 and H-5, typical of the majority of known pentalenolactone metabolites. The $^1$H NMR spectrum also revealed a pair of coupled doublets centered at δ 3.04 and 2.92 ($J$ = 4.7 Hz) resulting from the H-10 epoxide methylene protons. A pair of double doublets centered at δ 4.80 ($J$ = 6.0, 11.5 Hz, 1 H) and δ 4.18 ($J$ = 9, 11.5 Hz, 1 H) corresponded to the H-12 methylene protons coupled with the H-5 proton. From these data it was clear that the new metabolite was 22, the epoxide epimer of *epi*-pentalenolactone F methyl ester.

Table II. $^{13}$C NMR Spectral Data ($CDCl_3$) of the Methyl Esters of Pentalenolactone F (22) and *epi*-Pentalenolactone F (9-Me)

| C | 22 (ppm) | 9-Me (ppm) |
|---|---|---|
| 11 | 170.5 | 169.9 |
| 13 | 164.7 | 164.1 |
| 7 | 149.7 | 151.1 |
| 6 | 132.5 | 132.2 |
| 12 | 67.9 | 66.4 |
| 9 | 58.4 | 58.2 |
| 4 | 55.0 | 54.1 |
| 5 | 55.2[a] | 56.1[c] |
| 8 | 52.0[a] | 55.4[c] |
| 10 | 51.8[a] | 49.3 |
| 16 | 51.6 | 51.7 |
| 1 | 49.4 | 47.8 |
| 3 | 45.7 | 44.0 |
| 2 | 40.9 | 40.9 |
| 14 | 29.5[b] | 31.0[d] |
| 15 | 29.1[b] | 28.5[d] |

[a-d] Assignments with the same letter may be interchanged.

The stereochemistry of the epoxide moiety in 22 was unambiguously confirmed by $^1$H NOE experiments in combination with conformational modeling using the MACROMODEL program and an MM2 force field.[21] The lowest energy conformation of 22 was calculated by energy minimization after first constraining the dihedral angles of $H_5$–$C_5$–$C_{12}$–$H_{12\alpha}$ and $H_5$–$C_5$–$C_{12}$–$H_{12\beta}$ on the basis of the experimentally observed $^1$H NMR coupling constants ($J_{H\text{-}5,H\text{-}12}$) (Table III). In NOE experiments on pentale-

(20) A computer-generated representation of the molecular structure of 13 can be found in the supplementary material, along with detailed X-ray crystallographic data.

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**Table III. Observed and Calculated Coupling Constants for H-5, H-12 Protons of Pentalenolactone F (22), A (23), and B (24) Methyl Esters**

| compd | protons | obsd $J$ (Hz) | calcd $J$ (Hz) | calcd dihedral angle (deg) |
|---|---|---|---|---|
| **22** | H-12$\alpha$, H-5 | 9 | 10.9 | −157.1 |
| | H-12$\beta$, H-5 | 6 | 8.3 | −31.1 |
| **23** | H-12a, H-5 | 4.7 | 4.7 | −40.0 |
| | H-12b, H-5 | 4.8 | 4.6 | 40.3 |
| **24** | H-12a, H-5 | 5.1 | 5.1 | −37.3 |
| | H-12b, H-5 | 6.6 | 6.7 | 26.9 |

nolactone F methyl ester (**22**), the H-12$\alpha$ and H-12$\beta$ protons exhibited 0% and 5% NOE enhancements upon irradiation of H-5, respectively, while one of the H-10 signals at 2.92 showed a 3.3% NOE upon irradiation of H-3$\alpha$ (Figure 2). These results are consistent with the nonbonded proton–proton distances calculated by molecular modeling (Table IV). Additional NOEs consistent with the assigned configuration were observed between H-1$\beta$ and H-3$\beta$ and between H-5 and H-3$\beta$. By contrast, the observed NOEs corresponded poorly to the corresponding nonbonded distances calculated for the 9*S* epoxy diastereomer. (Data not shown.) The final dihedral angles in **22** derived from energy minimization were H–C(5)–C(12)–$H_\beta$ = −31.3° and H–C(5)–C(12)–$H_\alpha$ = −157.1°, corresponding to lactone conformation **B** (Figures 1 and 2).

Pentalenolactone F (**10**) may be derived biogenetically by epoxidation of pentalenolactone E (**6**) and is conceivably a precursor of pentalenolactone itself. On the other hand, formation of *epi*-pentalenolactone F (**9**) presumably occurs by epoxidation on the opposite face of the conjugated double bond of **9**. On the basis of stereochemical arguments, *epi*-pentalenolactone F (**9**) is unlikely to be a precursor of pentalenolactone. The factors which influence the stereochemical course of both the enzymatic and nonenzymatic oxidation[17] of the deoxy metabolite are not well understood and are clearly worthy of further study.

**Pentalenolactones A and B.** Pentalenolactone A methyl ester (**23**) (3 mg) and pentalenolactone B methyl ester (**24**) (2 mg) (Chart II) were isolated from a 4-L fermentation broth of *S.* UC5319 in relatively small amounts compared with the yield of pentalenolactone (65–70 mg).

Pentalenolactone A methyl ester (**23**) was isolated as an oily liquid. The high resolution mass spectrum corresponded to the molecular formula $C_{16}H_{18}O_5$. The infrared spectrum exhibited bands characteristic of a lactone (1785 $cm^{-1}$) and an ester (1714 $cm^{-1}$), with no hydroxyl group absorption, thereby accounting for four of the five oxygen atoms and suggesting the presence of four rings and an ether function. The structure of **23** was assigned on the basis of analysis of the $^1H$ and $^{13}C$ NMR and $^1H$ COSY NMR spectra. A characteristic triplet peak at $\delta$ 6.88 ($J$ = 2.3 and 2 Hz, H-7, 1 H) was evident in the $^1H$ NMR spectrum. A pair of double doublets centered at $\delta$ 4.75 ($J$ = 4.8, 11.8 Hz, 1 H) and $\delta$ 4.47 ($J$ = 4.7, 11.8 Hz, 1 H) corresponded to the H-12 methylene protons. The terminal epoxide residue was revealed by two doublets centered at $\delta$ 3.21 and 2.70 ($J$ = 4.6 Hz, H-10, 2 H). The $^{13}C$ NMR spectrum supported the presence of two double bonds, C-1 and C-2 ($\delta$ 130.4 and $\delta$ 129.7), C-6 ($\delta$ 133.7), and C-7 ($\delta$ 145.7) and also showed the presence of vicinal methyl groups (C-14 and C-15, $\delta$ 13.6 and 12.8). The $^1H$ NMR spectrum showed these methyl groups at $\delta$ 1.59 and 1.54 (H-14 and H-15, singlets, 6 H).

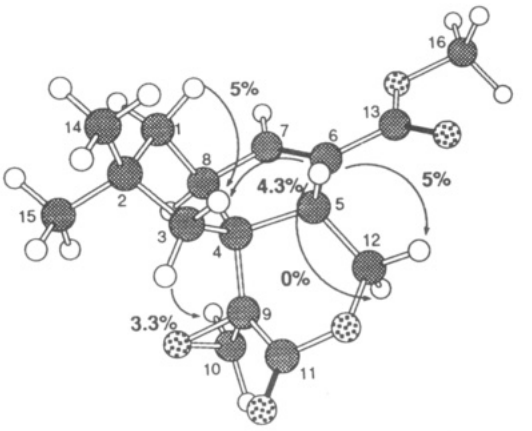


**Figure 2.** Pentalenolactone F methyl ester (**22**). The conformation was calculated by molecular mechanics and the arrows illustrate the most relevant experimental NOE effects.

The epoxide stereochemistry and lactone ring conformation of pentalenolactone A methyl ester were assigned by a combination of NOE and MM2 energy minimization studies. The nonbonded distances were calculated (Table IV) for the lowest energy conformation of pentalenolactone A methyl ester based on initially constraining the dihedral angles of $H_5$–$C_5$–$C_{12}$–$H_{12a}$ and $H_5$–$C_5$–$C_{12}$–$H_{12b}$ to correspond to the experimentally observed NMR coupling constants ($J_{H\text{-}5,H\text{-}12}$) (Table III). Irradiation of the H-5 proton gave rise to NOE enhancements for three protons: both H-12a (0.5%) and H-12b (2.5%) as well as H-3$\beta$ (5%). The corresponding MM2-calculated nonbonded distances for H-5–H-12a and H-5–H-12b were 2.44 and 2.43 Å while the distance for H-5–H-3$\beta$ was 2.32 Å (Table IV). The final dihedral angles for H–C(5)–C(12)–$H_a$ and H–C(5)–C(12)–$H_b$ were calculated to be −40° and 40°, respectively (Table III), corresponding to the gauche lactone conformation **A** (Figures 1 and 3). When the H-10b proton was irradiated, a 4% enhancement of the H-8 signal and 6.6% enhancement of the H-3$\alpha$ signal were observed. The corresponding nonbonded distances for H-10b–H-3$\alpha$ and H-10b–H-8 were 2.46 and 2.54 Å. The result established

**Table IV. Calculated Nonbonded Distances for Pentalenolactone F (22), A (23), and B (24) Methyl Esters[a]**

| 22 | | 23 | | 24 | |
|---|---|---|---|---|---|
| proton | nonbonded distance (Å) | proton | nonbonded distance (Å) | proton | nonbonded distance (Å) |
| H-3$\beta$, H-5 | 2.197 | H-3$\beta$, H-5 | 2.318 | H-3$\beta$, H-5 | 2.663 |
| H-3$\alpha$, H-10b | 4.323 | H-3$\alpha$, H-10b | 2.456 | H-3$\alpha$, H-10b | 2.421 |
| H-10b, H-8 | 2.188 | H-10b, H-8 | 2.539 | H-3$\alpha$, H-10a | 4.004 |
| H-1$\beta$, H-3$\beta$ | 2.915 | H-5, H-12a | 2.443 | H-10b, H-8 | 2.558 |
| H-5, H-12$\alpha$ | 3.109 | H-5, H-12b | 2.434 | H-5, H-12a | 2.127 |
| H-5, H-12$\beta$ | 2.397 | H-8, H-7 | 2.627 | H-5, H-12b | 2.804 |
| H-8, H-7 | 2.629 | H-5, H-10b | 4.996 | H-8, H-7 | 2.586 |
| H-8, H-3$\alpha$ | 3.648 | H-8, H-10a | 3.487 | H-5, H-10b | 5.100 |
| H-8, H-10a | 3.851 | | | H-8, H-1 | 2.307 |
| H-5, H-10b | 4.924 | | | H-8, H-3$\alpha$ | 3.157 |

[a] Calculated by Macromodel using the MM2 forcefield after constraining H(5)–C(5)–C(12)–H(12a) and H(5)–C(5)–C(12)–H(12b) dihedral angles based on observed coupling constants (see Table III). The most informative NOEs are illustrated in Figures 2–4.

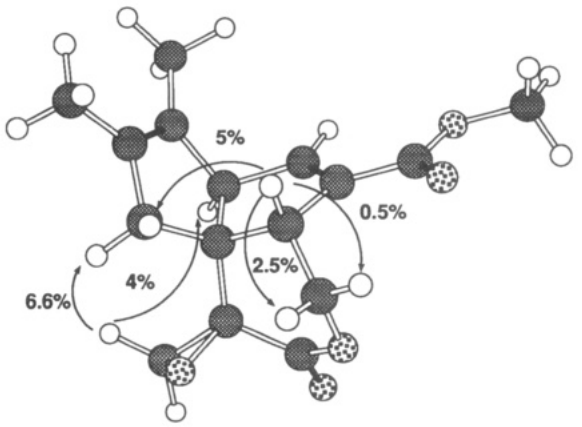


**Figure 3.** Pentalenolactone A methyl ester (**23**). The conformation was calculated by molecular mechanics and the arrows illustrate the most relevant experimental NOE effects.

the presence of a 9*R* β-epoxide, in spite of the fact that the lactone ring exists in conformation **A**.

Pentalenolactone B methyl ester (**24**) was also a thick oily liquid, and its molecular formula was assigned to be $C_{16}H_{18}O_5$ on the basis of exact mass. $^1H$ NMR and $^1H$ COSY together with $^{13}C$ NMR spectroscopic data revealed the partial structure of **24**. The characteristic chemical shifts of the H-15 methylene protons at δ 4.87 (singlet, H-15a, 1 H) and δ 4.71 (singlet, H-15b, 1 H) implied a double bond connecting C-15 and C-2. The $^{13}C$ NMR spectrum also supported the presence of an exomethylene double bond, C-2 (δ 145.1) and C-15 (δ 106.1). The epoxide residue exhibited two doublets centered at δ 3.18 ($J$ = 4.64 Hz, H-10a, 1 H) and δ 2.98 ($J$ = 4.64 Hz, H-10b, 1 H). The H-12 methylene protons appeared as a pair of double doublets centered at δ 4.75 ($J$ = 4.8, 7.1 Hz, 1 H) and δ 4.47 ($J$ = 4.7, 7.1 Hz, 1 H).

Irradiation of the H-5 proton resulted in NOE enhancements of the signals for both H-12a (5%) and H-12b (3%). The nonbonded distances for H-5–H-12a and H-5–H-12b were calculated by molecular mechanics to be 2.13 and 2.80 Å (Table IV), with the corresponding dihedral angles for H–C(5)–C(12)–$H_a$ = −37° and H–C(5)–C(12)–$H_b$ = 27° (Table III). Pentalenolactone B methyl ester is therefore also in the gauche lactone conformation **A**. The β-configuration of the C-14 methyl group was established by the strong (5%) NOE enhancement of the H-1 resonance observed upon irradiation of H-8. The H-3α and H-8 protons exhibited 5% and 6% NOE enhancements, respectively, upon irradiation of H-10b. The H-10b proton was thus shown to lie between H-3α and H-8. These results indicated that **24** contained a β-epoxide attached to a lactone ring in conformation **A** (Figures 1 and 4).

## Discussion

Pentalenolactones E, G, A, B, D, F, P, and O, *epi*-pentalenolactone F, pentalenic acid, and pentalenene as well as pentalenolactone itself have been isolated from the fermentation broth of *S.* UC5319. Pentalenolactone and pentalenic acid were produced as major components, while the other pentalenolactones were isolated as minor constituents, usually 4–7% that of pentalenolactone (Table V).

The structure of pentalenolactone D (**7**) was assigned by NMR spectroscopy and the stereochemistry was established by X-ray crystallography. For pentalenolactones F (**10**), A (**17**), and B (**18**), the structures were determined primarily by $^1H$ and $^{13}C$ NMR analysis. For the latter three metabolites, the major stereochemical problem in-

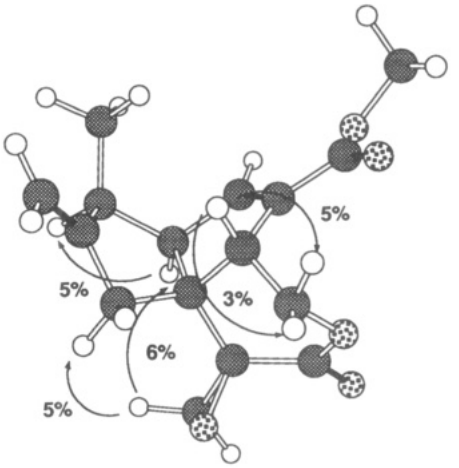


**Figure 4.** Pentalenolactone B methyl ester (**24**). The conformation was calculated by molecular mechanics and the arrows illustrate the most relevant experimental NOE effects.

**Table V. Yield of Pentalenolactones Produced by a 4-L Fermentation of *Streptomyces* UC5319**

| metabolite | mg |
|---|---|
| pentalenolactone methyl ester (**16-Me**) | 65 |
| pentalenic acid methyl ester (**5-Me**) | 25 |
| pentalenolactone A methyl ester (**23**) | 3 |
| pentalenolactone B methyl ester (**24**) | 2 |
| pentalenolactone D methyl ester (**20**) | 8 |
| pentalenolactone F methyl ester (**22**) | 3 |
| pentalenolactone E methyl ester (**6-Me**) | 3 |
| *epi*-pentalenolactone F methyl ester (**9-Me**) | 2 |
| pentalenolactone O methyl ester (**19-Me**) | 3–4 |
| pentalenolactone P methyl ester (**14-Me**) | 2 |

volved the assignment of the epoxide configuration. Although a set of empirical rules had suggested that the conformation of the lactone ring, as determined by the H-5–H-12 coupling constants, might be correlated with the configuration of the epoxide at C-9,[18] we used difference NOE measurements in combination with molecular mechanics to deduce the configuration of the epoxide moiety in each case. Thus an initial conformation for the lactone ring could be assigned on the basis of the observed NMR coupling constants for the H-5–H-12a and H-5–H-12b proton pairs and the lowest energy conformation calculated for each epoxide diastereomer. By comparing the calculated proton–proton distances with the observed NOEs, it was possible to assign the epoxide stereochemistry unambiguously. The molecular modeling indicated that for the 9*R* epoxide diastereomer of each metabolite, one of the H-10 protons lies below the bicyclo[3.3.0]octene ring system, giving rise to nonbonded NOE interactions with H-3α (pentalenolactone F) or both H-3α and H-8 (pentalenolactones A and B). By contrast, these interactions are absent in the corresponding 9*S* epoxide epimer.[22] Although the absolute configurations of each metabolite were not directly determined, these have been assigned on the basis of the co-occurrence of biogenetically and structurally related members of the pentalenolactone family of metabolites of known absolute configuration. Finally, it is worth pointing out that while pentalenolactone F conforms to the empirical rule correlating the more common 9*R* β-epoxide configuration with lactone conformation **B**, both pentalenolactones A and B have the same 9*R* β-epoxide configuration *and* the less common gauche lactone conformation **A**. Indeed, pentalenolactone itself would also

(22) See, for example, the computer-generated X-ray crystal structure of the methyl ester of *epi*-pentalenolactone F (**10**).[16b]

appear to exist in lactone conformation A, as inferred from coupling constant data ($J_{H\text{-}5,H\text{-}12}$ = 2.0, 4.16 Hz),[23] The conformation of the lactone ring therefore apparently depends not only on the configuration of the epoxide moiety but also on the nature of the substituents at C-1, as previously pointed out by the Hokkaido group.[18]

Pentalenolactone (16) can be derived from the hydrocarbon pentalenene (2) by a series of oxidation and methyl migration steps (Scheme I). The enzyme catalyzing the formation of pentalenene from farnesyl diphosphate (1), pentalenene synthase, has been purified to homogeneity[6b] and the stereochemistry and mechanism of pentalenene biosynthesis are now well understood as a result of extensive investigations.[7,8] Labeled pentalenene has also been incorporated into pentalenolactone (16), pentalenolactone E (6), *epi*-pentalenolactone F (9), and pentalenic acid (5) by feeding to intact cells.[8] Analysis of the labeling patterns in the derived metabolites indicated that pentalenic acid (5) and pentalenolactone H (3) could not serve as precursors of pentalenolactone.[8a]

A biosynthetic pathway from pentalenene to pentalenolactone can be proposed on the basis of earlier labeling experiments and the isolation of the four new metabolites pentalenolactone A, B, D, and F (Scheme I). Pentalenene (2), generated by enzyme-catalyzed cyclization of FPP (1), can be converted to deoxypentalenic acid (3). Although 3 has not been isolated to date as the free acid, Takahashi et al.[14] have isolated the corresponding deoxypentalenylglucuron (8) from *S. omiyaensis*. 3 may be further transformed by hydroxylation and lactonization. In the conversion of 3 to pentalenic acid (5), the net retention of configuration which has been demonstrated for the C-1 hydroxylation is consistent with the known course of numerous biological oxidations at unactivated methylene groups.[24] Pentalenic acid could then undergo a series of oxidative transformations to yield pentalenolactone H (3), which would under further oxidation to give pentalenolactone G (11).

Formation of pentalenolactone (16) from deoxypentalenic acid (3) would presumably require a second pathway involving oxidative cleavage of ring C of 3 to give pentalenolactone D (7). Dehydrogenation of 7 could then generate pentalenolactone E (6) which can undergo epoxidation to produce either of the two diastereomeric epoxide metabolites, pentalenolactone F (10), on the main biosynthetic pathway, or the shunt metabolite *epi*-pentalenolactone F (9).

We have proposed that the formation of the rearranged skeleton of pentalenolactone results from the generation of a positive charge at C-1 of an intermediate such as 13, followed by sequential migration of the adjacent β-methyl group (C-14) and loss of a proton from C-3.[8a] The generation of the secondary carbocation may occur directly by oxidative removal of H-1*si* from pentalenolactone F (10). Conceivably, the same C-1 cation might be generated by conversion of pentalenolactone F to the hypothetical intermediate 1-*epi*-pentalenolactone H (12), followed by protonation or other activation of the newly formed hydroxyl group.[25] Alternatively, the tertiary cation 15 might be generated by protonation of the cyclopropane ring of pentalenolactone P (14), itself derived from 13 by ionization of the hydroxyl group of 12 and insertion of the resultant cation into the adjacent β-methyl group. From cation 15, pentalenolactone A (17) could be formed by loss of the H-1 proton (pathway a), while pentalenolactone B (18) could be formed by loss of a proton from H-15 (pathway b). Finally elimination of H-3α from 15 would lead to the characteristic A-ring substitution pattern of pentalenolactone (16) itself (pathway c). Further investigation of this intriguing metabolic grid is in progress.

(23) The X-ray crystal structure of the bromohydrin derivative of tetrahydropentalenolactone also shows the lactone ring to be in gauche conformation A.[3]

(24) Hayaishi, O. *Oxygenases*; Academic Press: New York, 1962. Gautier, A. E. Dissertation, ETH Zurich, 1980, No. 6583.

(25) The methyl ester of 12 is a known compound, being the major product obtained by reduction of pentalenolactone G methyl ester.[10b,11]

## Experimental Section

**Small-Scale Fermentation of *S.* UC5319.** Mycelium from *S.* UC5319 on maltose-tryptone agar slants was transferred to a 500-mL DeLong flask with a Morton closure containing 100 mL of sterile vegetative medium, consisting of Pharmamedia (2.5 g) and Bactodextrose (2.5 g) in 100 mL of Nanopure water (100 mL), pH adjusted to 7.2. The flask was allowed to shake at 300 rpm at 28 °C for 2.5 days. A 1.0-mL portion of the vegetative culture was used to inoculate 24 500-mL flasks, each containing 100 mL of production medium consisting of NaCl (2 g), CaCO3 (5 g), corn gluten meal (10 g), Bactodextrose (1.125 g), blackstrap molasses (2 g), and corn starch (20 g) per liter of nanopure water adjusted to pH 7.2. After incubation at 28 °C with agitation (300 rpm) for 3 days, the culture medium was filtered by suction through a canvas pad. The mycelium was washed with water (2×) and the broth was acidified to pH 2.4 with 50% $H_2SO_4$ and extracted with chloroform. The organic layer was dried over sodium sulfate and concentrated to give a dark brown oil. This was dissolved in 50 mL of diethyl ether and the solution was filtered through a cotton plug which was washed with an additional 10 mL of diethyl ether.[7]

Evaporation of the ethereal solution provided an oil which was dissolved in 50 mL of 1:1 = THF:diethyl ether and treated with an ethereal solution of diazomethane (generated from *N*-nitroso-*p*-toluenesulfonamide in diethyl ether and a 50% KOH in water and ethanol solution) for 10 min at 0 °C. The reaction was quenched with a small amount of ethereal acetic acid, and the ether layer was washed twice with saturated sodium bicarbonate solution and once with brine. After being dried over sodium sulfate, the solution was concentrated to give a brown oil (300–400 mg). The mixture of methyl ester metabolites was purified by flash column chromatography on Merck Type H silica gel (10–40 μm) followed by MPLC, TLC, and HPLC, as described below. Thin layer chromatography (TLC) was performed using 20 × 20 cm Merck precoated glass-backed, silica gel 60 F-254 UV 0.25-mm plates for both preparative and analytical purposes. Visualization was by UV illumination, or the use of arsenomolybdate or *p*-anisaldehyde spray reagents followed by heating.

**Large-Scale Production of Metabolites of *S.* UC5319.** A fermentation culture containing 4 L of production medium in a 10-L cylindrical shake flask (autoclaved at 120 °C for 1 h) was inoculated with 50 mL of 2.5-day-old vegetative culture prepared as above. After being incubated for 3.5–4 days with aeration (8 psi, 6 liter/min) at 28 rpm, 32 °C, the culture broth was isolated by suction filtration through cloth. The mycelium was washed twice with water. The combined broths were acidified to pH 2.4 with 50% $H_2SO_4$ and then extracted with chloroform. The organic layer was dried over sodium sulfate and concentrated to give a dark brown oil. This oil was dissolved in 100 mL of diethyl ether, and the solution was filtered through a cotton plug which was washed with an additional 10 mL of diethyl ether. After esterification, the yield of crude methyl esters was 0.85–1.15 g per 4 L fermentation culture.

The metabolites obtained from a 10 liter cylindrical flask were initially purified by flash chromatography (silica gel, 5:1 = benzene:ethyl acetate) to provide a fraction ($R_f$ = 0.5) containing the methyl esters of pentalenolactone and pentalenolactone E, a fraction ($R_f$ = 0.45–0.4) containing the methyl esters of pentalenolactone F, pentalenolactone P, and pentalenolactone D, a fraction ($R_f$ = 0.4–0.30) containing the methyl esters of pentalenolactone A and pentalenolactone B, a fraction ($R_f$ = 0.25) containing the methyl ester of *epi*-pentalenolactone F and pentalenic acid, and a polar fraction ($R_f$ < 0.2) containing the methyl ester of pentalenolactone O.

**Isolation of Pentalenolactone F, Pentalenolactone P, and Pentalenolactone D Methyl Esters.** The fraction ($R_f$ =

0.45–0.4, benzene:ethyl acetate = 5.1) obtained by flash column chromatography (benzene:ethyl acetate = 10:1) was subjected to further purification by preparative thin layer chromatography (benzene:ethyl acetate = 25:1, 5 developments), resulting in the isolation of 8 mg of pentalenolactone D methyl ester (**20**) (least polar band, UV active). A second band, containing a mixture of pentalenolactone F and pentalenolactone P methyl esters was further purified by HPLC on a μ-Bondapak-CN column (Waters, 3.9 mm × 30 mm) using 30% ethyl acetate in hexane (1 mL/min) to give 3 mg of pentalenolactone F methyl ester (**22**) and 6 mg of pentalenolactone P methyl ester. Pentalenolactone D methyl ester (**20**): $^{13}C$ NMR ($CDCl_3$) δ 175.4, 164.4, 150.5, 132.6, 68.15, 57.56, 54.71, 51.72, 49.33, 44.87, 41.57, 39.87, 30.98, 28.98, 10.01; IR (neat) 2942, 2865, 2355, 2315, 1750, 1716, 1454, 1430, 1380, 1348 ($cm^{-1}$); $R_f$ = 0.42 (benzene:ethyl acetate = 5:1); CIMS $m/z$ ($NH_3$) found 279.1581 (calcd for $C_{16}H_{22}O_4$ [M + H] 279.1596). Pentalenolactone F methyl ester (**22**): IR (neat) 2953, 2919, 1768, 1712, 1398, 1318, 1259, 1187, 1158, 1107, 1097 ($cm^{-1}$); $R_f$ = 0.4 (benzene:ethyl acetate = 5:1); EIMS $m/z$ found 292.1280 (calcd for $C_{16}O_{20}O_5$ 292.1312).

**Pentalenolactone A and Pentalenolactone B Methyl Esters.** The fraction ($R_f$ = 0.4–0.30, benzene:ethyl acetate = 5:1) obtained by flash column chromatography was further purified by preparative thin layer chromatography (benzene–ethyl acetate, 30:1, five developments) to give 2 major UV-active bands. The less polar band was pentalenolactone A methyl ester (**23**) (3 mg) while the more polar band corresponded to pentalenolactone B methyl ester (**24**) (2 mg). Pentalenolactone A methyl ester (**23**): $^{13}C$ NMR ($CDCl_3$) δ 169.5, 164.5, 145.7, 133.2, 130.4, 129.7, 67.7, 65.3, 61.3, 58.2, 53.4, 51.8, 47.6, 46.3, 13.6, 12.8; IR (neat) 2916, 2850, 2358, 2333, 1762, 1712, 1437, 1387, 1350, 1266 ($cm^{-1}$); $R_f$ = 0.36 (benzene:ethyl acetate = 5:1); EIMS $m/z$ found 290.1140 (calcd for $C_{16}H_{18}O_5$ 290.1154). Pentalenolactone B methyl ester (**24**): $^{13}C$ NMR ($CDCl_3$) δ 169.5, 166, 151.73, 145.18, 135, 106.61, 67.71, 55.73, 53.41, 51.79, 51.31, 48.94, 48.91, 42.23, 40.39, 13.52; IR (neat) 2990, 2860, 1730, 1650, 1550, 1390, 1300 ($cm^{-1}$); $R_f$ = 0.34 (benzene:ethyl acetate = 5:1); CIMS $m/z$ ($NH_3$) found 290.1161 (calcd for $C_{16}H_{18}O_5$ 290.1149).

**Isolation and Purification of Phenacyl Esters of Pentalenolactones.** A 4-L culture of *S.* UC5319 was extracted as described above and the resulting brown oil (0.85 g) was dissolved in 100 mL of dry diethyl ether, filtered through a cotton plug which was rinsed with additional diethyl ether. Concentration of the solvent provided a brown oil which was dissolved in 50 mL of MeOH and was neutralized to pH 7.5 with a KOH/methanol (1 M) solution. The solvent was removed under vacuum to yield 600 mg of a mixture of potassium salts. A solution containing an excess of the alkylating agent, bromoacetophenone/18-crown-6 ether (20:1) was then added and the total volume of solution brought to 20 mL with acetonitrile. The solution was stirred at room temperature for 1 h and then filtered through 10 g of silica gel to remove crown ether and residual salts. The silica gel column was washed with benzene (40 mL) and the benzene eluent was concentrated in vacuo. The mixture of phenacyl esters was initially purified by flash column chromatography (10:1 = benzene:ethyl acetate) to provide a fraction ($R_f$ = 0.4–0.45, benzene:ethyl acetate = 6:1) containing pentalenolactone and pentalenolactone E phenacyl esters, a fraction ($R_f$ = 0.3, benzene:ethyl acetate = 6:1) containing pentalenolactone D phenacyl ester (**21**), a fraction ($R_f$ = 0.25, benzene:ethyl acetate = 6:1) containing pentalenic acid phenacyl ester, and a fraction ($R_f$ = 0.08, benzene:ethyl acetate = 6:1) containing pentalenolactone O phenacyl ester. The phenacyl esters of the various pentalenolactone metabolites could be further purified by preparative thin layer chromatography (benzene:ethyl acetate = 25:1, 6 developments) to provide pentalenolactone, pentalenolactone E, D, and O, phenacyl esters, and pentalenic acid phenacyl esters. Pentalenolactone D phenacyl ester (**21**) was recrystallized by vapor diffusion from pentane–THF. Pentalenolactone D phenacyl ester (2.5 mg) was transferred to a small tube and dissolved in THF (60 μL). Pentane (6 mL) was added to a larger tube and the small tube was placed in the large tube. The larger tube was then stoppered, left to stand for 2 days at room temperature, and then placed in a refrigerator for 2 days. The colorless crystals of pentalenolactone D phenacyl ester which were obtained were used for X-ray crystallographic structure determination. Pentalenolactone D phenacyl ester (**21**): $^1H$ NMR ($CDCl_3$) δ 7.91–7.47 (m, Ph, 5 H), 7.03 (t, $J$ = 2.24, 2.24 Hz, H-7, 1 H), 5.31 and 5.50 (AB q, $J$ = 16.4, $-COOCH_2CO-$, 2H), 4.88 (dd, $J$ = 7, 11.4 Hz, H-12α, 1 H), 4.08 (t, $J$ = 11.4, 11.4 Hz, H-12β, 1 H), 3.5 (m, H-5, 1 H), 3.20 (m, H-8, 1 H), 2.78 (q, $J$ = 7, H-9, 1 H), 1.82 (d, $J$ = 13.74 Hz, H-3α, 1 H), 1.69 (dd, $J$ = 10.4, 13.3 Hz, H-1α, 1 H), 1.56 (dd, $J$ = 1.69, 13.3 Hz, H-1β, 1 H), 1.42 (d, $J$ = 13.74 Hz, H-3β, 1 H), 1.19 (d, $J$ = 6.7 Hz, H-10, 3 H), 1.05 (s, H-14, 3 H), 1.03 (s, H-15, 3 H); IR (neat) 2964, 2866, 2360, 2338, 1750, 1734, 1456, 1394, 1266, 1183 ($cm^{-1}$); mp 158.5–159 °C; $R_f$ = 0.34 (benzene:ethyl acetate = 6:1).

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**Supplementary Material Available:** X-ray crystallographic data for pentalenolactone D (7 pages). Ordering information is given on any current masthead page.

# Enantioselective Synthesis of Calcium Channel Blockers of the Diltiazem Group

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A lipase-catalyzed kinetic resolution of racemic *trans*-2-phenylcyclohexanol readily provides the (−)-1*R*,2*S* enantiomer. This alcohol is employed as its chloroacetate **10a** in a chiral auxiliary-induced asymmetric Darzens glycidic ester condensation with *p*-anisaldehyde (**9**). Crystallization of the Darzens product affords enantiomerically pure (1*R*,2*S*)-2-phenylcyclohexyl (1*R*,2*S*)-2-(*p*-methoxyphenyl)glycidate (**11**), the structure of which was established by X-ray crystallography. The use of this glycidic ester in syntheses of diltiazem (**1**) and naltiazem (**8**), members of the diltiazem group of calcium channel blockers, provides these drug substances directly in enantiomerically pure form.

Calcium channel blockers inhibit the influx of $Ca^{2+}$ into vascular smooth muscle cells, thereby relaxing arteriolar smooth muscle and decreasing peripheral vascular resistance, with concomitant lowering of blood pressure.[1a] The

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