Caryophyllene Sesquiterpenoids from a Fungicolous Isolate of *Pestalotiopsis disseminata*

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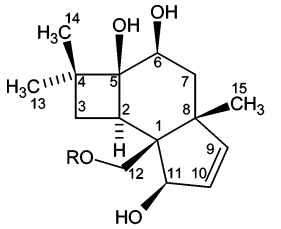
Three new caryophyllene-type sesquiterpene alcohols, 6-hydroxypunctaporonin E (**1**), 6-hydroxypunctaporonin B (**2**), and 6-hydroxypunctaporonin A (**3**), have been isolated from cultures of the fungicolous fungus *Pestalotiopsis disseminata*. The structures of **1**−**3** were determined mainly by analysis of 1D and 2D NMR data. The structure and absolute configuration of 6-hydroxypunctaporonin E (**1**) was confirmed through X-ray crystallographic analysis of its monobromobenzoate derivative. Compounds **1** and **2** showed activity against Gram-positive bacteria.

Explorations into the chemistry of fungi have shown them to be a plentiful source of a diverse array of natural products. Although fungal chemistry has been widely investigated, many ecological niche groups remain underexplored. One unique niche is occupied by fungicolous fungi, those that colonize other fungal species. Our chemical studies of fungicolous fungi have shown them to be rich sources of novel metabolites, even among genera that have been examined previously.[1−6]

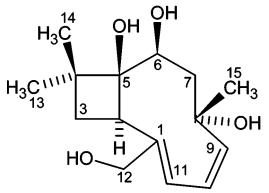
The genus *Pestalotiopsis* has been the subject of a handful of prior chemical studies. Chemical investigation of a phytopathogenic isolate of *P. oenotherae* yielded both polyketide and terpenoid constituents.[7] Studies of endophytic *Pestalotiopsis* spp. have yielded the caryophyllene-derived pestalotiopsins, pestalotiopsolides, and taedolidols.[8−10] Recently, our studies of a fungicolous isolate of *Pestalotiopsis disseminata* (Thuem.) Stey. (Amphisphaeriaceae; MYC-1444 = NRRL 36915) have afforded three new sesquiterpene alcohols named 6-hydroxypunctaporonin E (**1**), 6-hydroxypunctaporonin B (**2**), and 6-hydroxypunctaporonin A (**3**). Details of the isolation and structure determination of these compounds are presented here.

The punctaporonins are a set of six caryophyllene sesquiterpenoids that were originally isolated from the coprophilous fungus *Poronia punctata* (Linnaeus:Fries).[11−14] The structures of punctaporonins A−F were originally determined via ^{1}H NMR, MS, and X-ray crystallography, and the absolute configurations of punctaporonins A and D were determined by enantiospecific total synthesis.[15]



1 R = H

4 R = CO-*p*-C_6H_4Br



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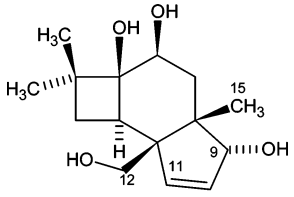

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Results and Discussion

Fractionation of an organic extract from solid-substrate fermentation of *P. disseminata* using silica gel, followed by purification employing reversed-phase HPLC, afforded compounds **1**−**3**. These sesquiterpene alcohols are new 6-hydroxy derivatives of punctaporonins E, B, and A, respectively.

The molecular formula for compound **1** was determined to be $C_{15}H_{24}O_4$ (four degrees of unsaturation) by analysis of ^{1}H and ^{13}C NMR data (Table 1) in conjunction with DEPT results, and this conclusion was confirmed by HRESIMS. The ^{13}C NMR data showed that compound **1** had only two olefinic carbons and no carbonyl groups and must therefore be tricyclic in order to account for the remaining degrees of unsaturation. DEPT and ^{1}H NMR data indicated that the olefin must be 1,2-disubstituted, and the small *J*-value (5.7 Hz) is consistent with the presence of a *cis*-olefin unit, most likely incorporated into a small ring. Comparison of the ^{1}H NMR and DEPT data with the molecular formula indicated that there must be four exchangeable protons, requiring the presence of four free hydroxy groups. Signals for four oxygenated carbons (one methylene, two methines, and a quaternary carbon) were observed, along with nine additional sp^3 signals (three quaternary carbons, one methine, two methylenes, and three methyl groups) in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum showed that all three methyl groups are isolated, as is the primary alcohol unit.

^{1}H NMR coupling information enabled identification of the C2−C3, C6−C7, and C9−C10−C11 units. HMBC data were used to establish the connectivity of these units with the remainder of the molecule. HMBC correlations from geminal methyls H_3-13 and H_3-14 to C-3, C-4, and C-5 extended the C2−C3 fragment through C-4 and C-5. Correlations were also observed from H_3-15 to C-1, C-7, C-8, and C-9 and from H-6 to C-5, linking the C6−C7 and C9−C10−C11 fragments via methylated quaternary carbon C-8. Key correlations between H_2-12 and C-1, C-2, C-8, and C-11 located the primary alcohol unit and enabled closure of the five- and six-membered rings in **1**. HMBC correlations from H-2 to C-11 and C-6 required the presence of a cyclobutane ring to complete

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Table 1. NMR Data for 6-Hydroxypunctaporonin E (**1**)

position	δ_H[a] (multiplicity, J_{HH})	δ_C[b]	HMBC[c] correlations (C#)	NOESY[d] correlations (H#)
1		50.3		
2	2.00 (dd, 12.8, 7.4)	42.9	*e*	3b, 6, 11, 13
3a	2.14 (dd, 12.8, 8.3)	35.8	*e*	2, 3a
3b	1.59 (dd, 8.3, 7.4)		1, 2, 4, 5, 12[f], 14	3b, 12b, 14
4		42.1		
5		80.3		
6	3.58 (dd, 10.5, 5.7)	69.3	5, 7	13
7a	1.80 (dd, 13.5, 5.7)	44.0	1, 5, 6, 8, 9, 15	15
7b	1.73 (dd, 13.5, 10.5)		1, 5, 6, 8, 9, 15	6, 9
8		53.3		
9	5.83 (d, 5.7)	147.4	1, 7, 8, 10, 11, 15	7b, 15
10	5.68 (dd, 5.7, 2.7)	129.8	1, 8, 9, 11, 15[f]	11
11	4.12 (d, 2.7)	81.6	2, 8, 9, 10	2, 10
12a	4.20 (d, 11.4)	61.4	1, 2, 11	3b, 12a
12b	3.83 (d, 11.4)		1, 2, 8, 11	12b, 15
13	1.12 (s)	24.2	3, 4, 5, 14	2, 6
14	1.13 (s)	23.3	3, 4, 5, 13	3a
15	1.24 (s)	27.9	1, 6[f], 7, 8, 9	7b, 9, 12a

[a] Recorded in $CDCl_3$ at 300 MHz. [b] Recorded in acetone-d_6 at 75 MHz. [c] Recorded in acetone-d_6 at 600 MHz (^{1}H dimension). [d] Recorded in $CDCl_3$ at 400 MHz. [e] Fortuitous overlap of these signals in acetone-d_6 prevented unambiguous assignment of their correlations. [f] Four-bond correlation.

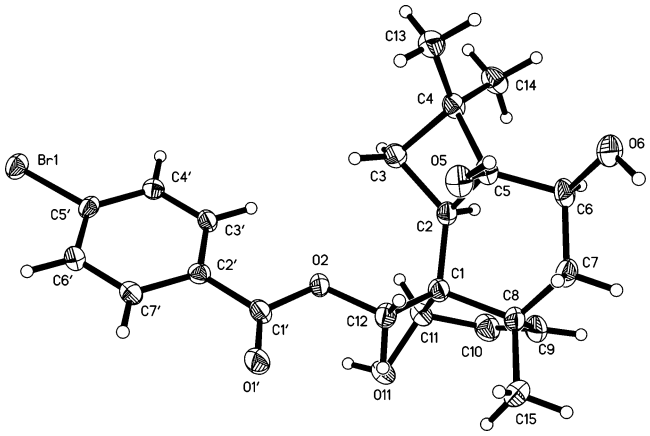

Figure 1. X-ray model of 12-(4-bromobenzoyl)-6-hydroxypunctaporonin E (**4**). Thermal ellipsoids are shown at the 30% level.

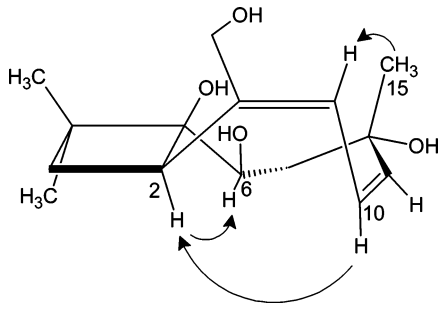

Figure 2. Selected NOESY correlations for 6-hydroxypunctaporonin B (**2**).

the ring system. This planar structure is similar to that of punctaporonin E, differing only in the presence of an additional hydroxy group at C-6. It was therefore assigned the trivial name 6-hydroxypunctaporonin E (**1**).

The relative configuration of **1** was determined via analysis of NOESY data (Table 1). The structure of **1** was confirmed and the absolute configuration assigned through single-crystal X-ray diffraction analysis of the mono-bromobenzoate derivative (**4**; Figure 1). The inclusion of a bromine atom in the molecule allowed determination of the absolute configuration as shown in **1** (1*R*, 2*R*, 5*S*, 6*S*, 8*R*, and 11*R*). These data allow for the tentative assignment of the absolute configuration of punctaporonin E by analogy.

Compound **2** is an isomer of **1**, but contains two additional sp^2 carbons, indicating the presence of an additional double bond and only two rings in **2**. In addition, one of the oxygenated methine signals observed for **1** was replaced by an additional oxygenated quaternary carbon signal in the ^{13}C NMR spectrum of **2**. ^{1}H−^{1}H coupling and HMBC data revealed the presence of the same C2−C7 structural unit found in **1**, as well as a new conjugated diene system corresponding to the C1/C9−C11 unit in **2**. HMBC correlations of H_3-15 to C-7, C-9, and the new oxygenated quaternary carbon (C-8) enabled connection of C-15, C-7, and C-9 to C-8. Correlations of H_2-12 to C-1, C-2, and C-11 led to completion of the gross structure as shown in **2**.

The relative configuration of **2** was assigned by analysis of NOESY data. As in **1**, H-2 correlated to H-6, requiring a *trans* ring fusion, and placing H-2 and H-6 on the same face of the ring system. H-10 correlated to H-2, thus requiring the *E*-geometry for the C-1/C-11 olefin, and H_3-15 correlated to H-11, requiring the *Z*-configuration for the C-9/C-10 olefin (Figure 2). The H_3-15 to H-11 correlation also permits the assignment of H_3-15 to the top face of the ring system. This relative configuration matches that of punctaporonin B.[12] The absolute configuration is presumed to be analogous to that of **1** (2*R*, 5*S*, 6*S*, and 8*S*). The difference in the *R*/*S* designation at position 8 is due to a change in priority assignments in **2** relative to **1**.

The NMR data for compound **3** were nearly identical to those of **1**, except that the HMBC data for **3** showed a correlation from H_3-15 to an oxygenated methine carbon (C-9) rather than an olefinic carbon, and H_2-12 correlated with an olefinic carbon (C-11) rather than an oxymethine carbon, indicating that the hydroxy and olefinic groups were transposed from their positions in **1**.

The NOESY spectrum of **3** closely resembled that of **1** (Table 1). H-2 showed correlations to H-3b, H-6, and H_3-13, placing all of these hydrogens on the same face of the ring system. H_2-12 showed correlations to H-3a and H_3-15, placing these protons on the opposite face of the ring system relative to H-2. Correlation of H-9 with H_3-15 indicated their *cis* relationship. Again, the absolute configuration is presumed to be analogous to that of **1** and punctaporonin A[15] (1*S*, 2*R*, 5*S*, 6*S*, 8*S*, and 9*R*).

The punctaporonins were originally characterized using 1D NMR techniques, and their structures were verified by X-ray crystallography. On the basis of the HMQC results obtained for **3**, the reported ^{1}H NMR data[11] for punctaporonin A appear to contain a minor misassignment. In the literature report, the proton at C-2 was assigned as one of the methylene protons of C-3. This assignment was understandably complicated by the fact that the geminal coupling constant between the protons on C-3 is only 7.6 Hz, while the vicinal coupling between the downfield C-3 proton

and the C-2 proton is 12 Hz. Similar HMQC correlations were observed in **1** and **2**, therefore resulting in a similar adjustment in the corresponding assignments for punctaporonins A, B, and E.

Literature descriptions of the biological activity of the punctaporonins have varied from "of little interest"[14] to "remarkable".[15] Compounds **1** and **2** exhibited activity in standard agar disk diffusion assays[16,17] at 100 μg/disk against *Bacillus subtilis* (ATCC 6051), each causing a 12-mm zone of inhibition. *Staphylococcus aureus* (ATCC 29213) was inhibited to a lesser extent by **1** and **2**, the zone being 8 mm in both cases. No activity was observed for **3** against *B. subtilis* or *S. aureus*. All three compounds were for inactive in assays against *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 14053) at 100 μg/disk or against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) at 200 μg/disk.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Rudolph Research Autopol III automatic polarimeter. UV measurements were performed with a Varian Cary 100 Bio UV−vis spectrophotometer. IR measurements employed a Perkin-Elmer Spectrum BX FT-IR instrument. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AC-300 or a DPX-300 spectrometer. NOESY data were recorded on a Bruker DRX-400. HMBC and HMQC experiments were performed on a Bruker AMX-600 spectrometer. Chemical shift values were referenced to residual solvent signals as follows (δ_H/δ_C): acetone-d_6 (2.05/29.8), $CDCl_3$ (7.24/77.0), CD_3OD (3.30/49.0). ESIMS and HRESIMS data were recorded on a Micromass Autospec instrument, and EIMS (70 eV) data were obtained using a Finnigan Voyager instrument.

Fungal Material. Stromata of an unidentified pyrenomycete growing on a dead hardwood branch in a hardwood swamp within Reed Bingham State Park, Adel, GA, were collected by B. W. Horn on April 29, 2000. The stromata, along with portions of the woody substrate on which the stromata had formed, were placed in plastic bags and stored at 5 °C. To isolate microfungal colonists, stromatal surfaces were gently abraded with a surface-sterilized fingernail file to remove portions of the blackened tissues. Direct plating of stromatal filings was accomplished by sprinkling a small portion (100−200 mg) of the filings over the surface of each of two plates of dextrose-peptone-yeast extract agar (DPYA) containing streptomycin (25 mg/L) and tetracycline (1.25 mg/L).[18] Plates were incubated in the dark at 25 °C for 5 days, and representative cultures were isolated from each colony type showing a distinctive morphology on DPYA. After 7−12 days incubation, the tube cultures isolated from the stromatal filings were segregated into groups of presumptive species and maintained for identification and rice fermentation (25 °C).

One of these cultures, MYC-1444, was determined by D.T.W. to be an isolate of *Pestalotiopsis disseminata* (Thuemen) Steyaert. A subculture of this organism was deposited in the ARS collection at the USDA National Center for Agricultural Utilization Research in Peoria, IL, with the accession number NRRL 36915.

Fermentation was carried out in a single 500-mL Erlenmeyer flask containing 50 g of rice. Distilled H_2O (50 mL) was added to the flask, and the contents were soaked overnight before autoclaving at 15 lb/in.^{2} for 30 min. The flask was cooled to room temperature, inoculated with 3.0 mL of spore inoculum, and incubated for 30 days at 25 °C. After incubation, the fermented rice substrate was mechanically fragmented and then extracted repeatedly with EtOAc (3 × 100 mL). The combined EtOAc extracts were filtered and evaporated to give a yellow oil (419 mg).

Isolation of 1−3. The EtOAc extract (419 mg) was partitioned between hexanes and CH_3CN. The CH_3CN phase (384 mg) was then fractionated on a silica gel column, eluting successively with 100% CH_2Cl_2, 98:2 CH_2Cl_2−CH_3OH, 95:5 CH_2Cl_2−CH_3OH, 90:10 CH_2Cl_2−CH_3OH, and 80:20 CH_2Cl_2−CH_3OH, and 20-mL fractions were collected. Compounds **1**, **2**, and **3** were present in the 15th fraction (74 mg), eluting at 90:10 CH_2Cl_2−CH_3OH. They were separated by preparative reversed-phase HPLC (Rainin Dynamax, C-18 column, 2.0 × 30 cm, 10 mL/min) using 20% CH_3CN in water for 25 min, followed by a linear gradient from 20 to 60% CH_3CN over 15 min to afford **1** (20 mg), **2** (9 mg), and **3** (12 mg).

6-Hydroxypunctaporonin E (1): white powder; $[\alpha]^{25}_D$ −20 (*c* 0.08, CH_3OH); HPLC t_R 14.7 min under the above conditions; UV (CH_3OH) λ_{max} (log ϵ) 201 (3.38); IR (KBr) ν_{max} 3401, 2918, 1630, 1384, 1095, 1046 cm^{-1}; ^{1}H NMR, ^{13}C NMR, HMBC, and NOESY data, see Table 1; EIMS (70 eV) *m*/*z* 250 (M − H_2O; rel int 2), 232 (3), 219 (5), 194 (42), 176 (12), 163 (16), 150 (49), 125 (100), 107 (53), 91 (62), 55 (75); HRESIMS *m*/*z* 267.1599 [M − H]$^-$, calcd for $C_{15}H_{23}O_4$, 267.1596.

6-Hydroxypunctaporonin B (2): colorless glass; $[\alpha]^{25}_D$ −80 (*c* 0.08, CH_3OH); HPLC t_R 28.5 min under the above conditions; UV (CH_3OH) λ_{max} (log ϵ) 198 (3.62) 206 (3.51); IR (KBr) ν_{max} 3414, 2932, 1705, 1642, 1384, 1120, 1065 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.87 (1H, d, J = 2.2 Hz, H-11), 5.72 (1H, d, J = 13 Hz, H-9), 5.63 (1H, ddd, J = 13, 2.2, 2.1 Hz, H-10), 4.12 (1H, dd, J = 11, 2.1 Hz, H-12a), 4.02 (1H, d, J = 4.8 Hz, H-6), 3.88 (1H, d, J = 11 Hz, H-12b), 3.35 (1H, dd, J = 12, 8.2 Hz, H-2), 2.82 (1H, dd, J = 16, 4.8 Hz, H-7a), 2.15 (1H, dd, J = 12, 10 Hz, H-3a), 1.54 (1H, dd, J = 10, 8.2 Hz, H-3b), 1.41 (1H, d, J = 16 Hz, H-7b), 1.26 (3H, s, H_3-13), 1.24 (3H, s, H_3-15), 1.10 (3H, s, H_3-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.2 (d, C-9), 138.2 (s, C-1), 127.8 (d, C-11), 124.4 (d, C-10), 82.7 (s, C-5), 74.2 (s, C-8), 71.8 (d, C-6), 65.1 (t, C-12), 46.9 (t, C-7), 40.4 (s, C-4), 39.2 (d, C-2), 33.7 (t, C-3), 32.5 (q, C-15), 24.6 (q, C-13), 23.8 (q, C-14); HMBC (acetone-d_6, 600 MHz) H-2 → C-1, 3, 4, 5, 6, 11, 12; H-3a → C-1, 2, 4, 5, 13, 14; H-3b → C-2, 4, 5, 6, 13; H-6 → C-8; H-7a → C-5, 6, 8, 9, 15; H-7b → C-5, 6, 8, 9, 15; H-9 → C-1, 7, 11; H-10 → C-1, 8; H-11 → C-1, 2, 5, 9, 10, 12; H-12a → C-1, 2, 11; H-12b → C-1, 2, 11; H_3-13 → C-3, 4, 5, 14; H_3-14 → C-3, 4, 5, 13; H_3-15 → C-7, 8, 9; NOESY (acetone-d_6, 400 MHz) H-2 → H-3b, 6, 10, 13; H-3a → H-3b, 12a, 12b, 14; H-3b → H-2, 3a, 12a, 12b, 13; H-6 → H-2, 7a, 7b, 13; H-7a → H-6, 7b, 11, 15; H-7b → H-6, 7a; H-9 → H-15; H-10 → H-2; H-11 → H-7a, 12b, 15; H-12a → H-3a, 12b; H-12b → H-3a, 11, 12a; H_3-13 → H-2, 6; H_3-14 → H-3a; H_3-15 → H-7a, 9, 11; EIMS (70 eV) *m*/*z* 250 (M − H_2O; rel int 1), 232 (4), 217 (4), 204 (18), 175 (9), 161 (16), 147 (17), 131 (48), 107 (60), 91 (83), 55 (100); HRESIMS *m*/*z* 267.1594 [M − H]$^-$, calcd for $C_{15}H_{23}O_4$, 267.1596.

6-Hydroxypunctaporonin A (3): white powder; $[\alpha]^{25}_D$ −11 (*c* 0.07, CH_3OH); HPLC t_R 18.1 min under the above conditions; UV (CH_3OH) λ_{max} (log ϵ) 202 (3.38); IR (KBr) ν_{max} 3394, 2926, 1632, 1384, 1088, 1020 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.70 (2H, br s, H-10 and H-11), 4.18 (1H, dd, J = 9.7, 5.2 Hz, H-6), 4.07 (1H, s, H-9), 3.69 (1H, d, J = 10.5 Hz, H-12a), 3.46 (1H, d, J = 10.5 Hz, H-12b), 2.38 (1H, dd, J = 12, 7.5 Hz, H-2), 2.06 (1H, dd, J = 12, 7.6 Hz, H-3a), 1.91 (1H, dd, J = 13, 5.2 Hz, H-7a), 1.63 (1H, dd, J = 13, 9.7 Hz, H-7b), 1.53 (1H, dd, J = 7.6, 7.5 Hz, H-3b), 1.19 (3H, s, H_3-13), 1.12 (3H, s, H_3-14), 1.02 (3H, s, H_3-15); ^{1}H NMR data for **3** were recorded in both acetone-d_6 and CD_3OD because the olefinic signals were fortuitously overlapping in CD_3OD, but acetone-d_6 obscured the signal at δ 2.06; ^{13}C NMR (CD_3OD, 75 MHz) δ 142.3 (d, C-11), 130.6 (d, C-10), 88.4 (d, C-9), 81.1 (s, C-5), 70.1 (d, C-6), 62.6 (t, C-12), 55.6 (s, C-1), 50.9 (s, C-8), 48.1 (d, C-2), 43.1 (s, C-4), 41.0 (t, C-7), 35.9 (t, C-3), 24.7 (q, C-15), 24.3 (q, C-14), 23.5 (q, C-13); HMBC (acetone-d_6, 600 MHz) H-2 → C-1, 3, 4, 5, 6, 8, 11, 12; H-3a → C-1, 2, 4, 5, 13, 14; H-3b → C-2, 4, 5, 6, 13, 14; H-6 → C-5, 7; H-7a → C-1, 4, 5, 6, 8, 9, 15; H-7b → C-5, 6, 8, 9, 15; H-9 → C-1, 10, 11, 15; H-10 → C-1, 8, 9, 11; H-11 → C-1, 8, 9, 10, 12; H-12a → C-1, 2, 8, 11; H-12b → C-1, 2, 8, 11; H_3-13 → C-3, 4, 5, 14; H_3-14 → C-3, 4, 5, 13; H_3-15 → C-1, 7, 8, 9; NOESY ($CDCl_3$, 400 MHz) H-2 → H-3b, 6, 13; H-3a → H-3b, 12a, 14; H-3b → H-3a; H-6 → H-2, 7a, 13; H-7a → H-6, 7b, 15; H-7b → H-7a, 15; H-9 → H-7a, 15; H-12a → H-3a, 12b; H-12b → H-12a, 15; H_3-13 → H-6; H_3-14 → H-3a; H_3-15 → H-7a, 7b, 9, 12b; overlap of H-10 and H-11 prevented unambiguous assignment of NOESY correlations for those signals; EIMS (70 eV) *m*/*z* 250 (M − H_2O; rel int 2), 237 (23), 219 (18), 194 (37), 181 (90), 163 (100), 145 (49), 135 (78), 107 (57), 91 (92), 55 (98); HRESIMS *m*/*z* 267.1587 [M − H]$^-$, calcd for $C_{15}H_{23}O_4$, 267.1596.

12-(4-Bromobenzoyl)-6-hydroxypunctaporonin E (4): A sample of **1** (6.0 mg) was dissolved in dry THF and added to a 10-mL round-bottom flask. To the flask were added 2.7 mg of DMAP and 5.4 mg of 4-bromobenzoyl chloride. Three milliliters of dry CH_2Cl_2 was added to aid in the dissolution of the reagents. The reaction vessel was sealed under an Ar atmosphere and stirred at room temperature for 168 h. Purification was carried out by reversed-phase HPLC (Beckman Ultrasphere, semipreparative C-18 5-μm column, 1.0 × 25 cm, 2 mL/min) with a linear gradient from 20 to 100% CH_3CN in water over 40

min. The desired monoacylation product eluted at 27.4 min: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88 (2H, br d, J = 8.7 Hz), 7.58 (2H, br d, J = 8.7 Hz), 5.83 (1H, d, J = 5.6 Hz, H-9), 5.79 (1H, dd, J = 5.6, 2.6 Hz, H-10), 5.22 (1H, d, J = 10.6 Hz, H-12a), 4.77 (1H, dd, J = 10.6, 1.3 Hz, H-12b), 4.32 (1H, br s, H-11), 3.65 (1H, dd, J = 9.8, 6.4 Hz, H-6), 2.24 (1H, dd, J = 12, 9.0 Hz, H-3a), 1.92 (1H, dd, J = 12, 7.9 Hz, H-2), 1.86 (1H, dd, J = 14, 9.8 Hz, H-7a), 1.80 (1H, dd, J = 14, 6.4 Hz, H-7b), 1.69 (1H, dd, J = 9.0, 7.9 Hz, H-3b), 1.14 (3H, s, H_3-15), 1.10 (3H, s, H_3-14), 1.05 (3H, s, H_3-13); ESIMS m/z 473 [M + Na]$^+$.

X-ray Crystallographic Analysis of 12-(4-Bromobenzoyl)-6-hydroxypunctaporonin E (4).[19] A colorless needle (0.32 × 0.03 × 0.03 mm) obtained upon crystallization from 7:3 CH_3CN-H_2O was separated from the sample, mounted with grease on the tip of a glass capillary epoxied to a brass pin, and placed on the diffractometer with the long crystal dimension (unit cell *a*-axis) approximately parallel to the diffractometer phi axis. Data were collected on a Nonius KappaCCD diffractometer (Mo Kα radiation, graphite monochromator) at 190(2) K (cold N_2 gas stream) using standard CCD techniques, yielding 29 073 data. Lorentz and polarization corrections were applied. Equivalent data were averaged, yielding 3964 unique data (R_{int} = 0.069, 3134 $F > 4\sigma(F)$). Cell dimensions were determined to be a = 6.7816(7) Å, b = 16.7520(17) Å, c = 19.939(2) Å, α = 90°, β = 90°, γ = 90°. On the basis of a preliminary examination of the crystal, the space group $P2_12_12_1$ was assigned (no significant exceptions to the systematic absences $h00$, h = odd; $0k0$, k = odd; $00l$, l = odd were noted). The computer programs from the HKL package were used for data reduction. The preliminary model of the structure was obtained using XS, a direct methods program. Least-squares refining of the model versus the data was performed with the XL computer program. Illustrations were made with the XP program, and tables were made with the XCIF program. All are in the SHELXTL v6.1 package. Thermal ellipsoids shown in the illustration are at the 30% level. All non-hydrogen atoms were refined with anisotropic thermal parameters, and the final refinement gave R_1 = 0.0400, wR_2 = 0.0736 (refinement on F^2). All hydrogen atoms were included with the riding model using the XL program default values. A CH_3CN molecule of solvation was included in the crystal structure. It was refined as a rigid group (C−N = 1.1 Å, C−C = 1.5 Å, C−H = 0.99 Å, N−C−C = linear, C−C−H = tetrahedral) with an isotropic thermal parameter and occupancy = 0.415(7). No further restraints or constraints were imposed on the refinement model.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of **1**−**3** and ^{1}H NMR data for **4** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Li, C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *J. Nat. Prod.* **2005**, *68*, 319−322.
(2) Angawi, R. F.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2005**, *68*, 212−216.
(3) Che, Y.; Gloer, J. B.; Wicklow, D. T. *Org. Lett.* **2004**, *6*, 1249−1252.
(4) Zhang, Y.; Li, C.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Org. Lett.* **2003**, *5*, 773−776.
(5) Soman, A. G.; Angawi, R. F.; Gloer, J. B.; Wicklow, D. T. *J. Nat. Prod.* **2001**, *64*, 189−192.
(6) Li, C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Org. Lett.* **2002**, *4*, 3095−3098.
(7) Venkatasubbaiah, P.; Van Dyke, C. G.; Chilton, W. S. *Phytochemistry* **1991**, *30*, 1471−1474.
(8) Pulici, M.; Sugawara, F.; Koshino, H.; Uzawa, J.; Yoshida, S.; Lobkovsky, E.; Clardy, J. *J. Org. Chem.* **1996**, *61*, 2122−2124.
(9) Pulici, M.; Sugawara, F.; Koshino, H.; Okada, G.; Esumi, Y.; Uzawa, J. *Phytochemistry* **1997**, *46*, 313−319.
(10) Magnani, R. F.; Rodrigues-Fo, E.; Daolio, C.; Ferreira, A. G.; de Souza, A. Q. *Z. Naturforsch.* **2003**, *58*, 319−324.
(11) Anderson, J. R.; Edwards, R. L.; Poyser, J. P.; Whalley, A. J. *J. Chem. Soc., Perkin Trans. 1* **1988**, 823−831.
(12) Anderson, J. R.; Briant, C. E.; Edwards, R. L.; Mabelis, R. P.; Poyser, J. P.; Spencer, H.; Whalley, A. J. *J. Chem. Soc., Chem. Commun.* **1984**, 405−406.
(13) Anderson, J. R.; Edwards, R. L.; Freer, A. A.; Mabelis, R. P.; Poyser, J. P.; Spencer, H.; Whalley, A. J. *J. Chem. Soc., Chem. Commun.* **1984**, 917−919.
(14) Poyser, J. P.; Edwards, R. L.; Anderson, J. R.; Hursthouse, M. B.; Walker, N. P.; Sheldrick, G. M.; Whalley, A. J. *J. Antibiot.* **1986**, *39*, 167−169.
(15) Sugimura, T.; Paquette, L. A. *J. Am. Chem. Soc.* **1987**, *109*, 3017−3024.
(16) Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493−496.
(17) Wagenaar, M. M.; Clardy, J. *J. Nat. Prod.* **2001**, *64*, 1006−1009.
(18) Papavizas, G. C.; Davies, C. B. *Soil Sci.* **1959**, *88*, 112−117.
(19) Crystallographic data for compound **4** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 284689). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033; or e-mail: deposit@ccdc.cam.ac.uk).

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