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Botryolides A−E, Decarestrictine Analogues from a Fungicolous *Botryotrichum* sp. (NRRL 38180)[⊥]

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Four new decarestrictine analogues (botryolides A−D; **1**–**4**), a biosynthetically related γ-lactone (botryolide E; **5**), and the known compounds decarestrictine D (**6**) and sterigmatocystin have been isolated from cultures of a fungicolous isolate of *Botryotrichum* sp. (NRRL 38180). The structures of these compounds were determined by analysis of 2D NMR and ESIMS data. The relative configurations of **1**–**5** were established on the basis of NMR data and/or X-ray diffraction analysis, while the absolute configuration of **1** was assigned using the modified Mosher method.

Mycoparasitic and fungicolous fungi are those that colonize and/or parasitize other fungi. We have targeted such organisms as potential sources of new compounds with antifungal effects and other bioactivities.[1−4] During our ongoing studies in this area, four new decarestrictine analogues named botryolides A−D (**1**–**4**), another polyketide metabolite of apparently similar origin (botryolide E; **5**), and the known compounds decarestrictine D (**6**) and sterigmatocystin were obtained from an isolate of the *Botryotrichum* state of *Chaetomium piluliferum* (MYC-1117 = NRRL 38180; Chaetomiaceae) obtained from the surface of a polypore collected in Florida. Details of the isolation and structure elucidation of these metabolites are presented here.

Results and Discussion

The ethyl acetate extract of solid-substrate fermentation cultures of MYC-1117 showed potent activity against the fall armyworm *Spodoptera frugiperda* and was selected for chemical investigation. The extract was partitioned between hexanes and acetonitrile, and the acetonitrile-soluble fraction was separated by silica gel column chromatography and reversed-phase HPLC to afford botryolides A−E (**1**–**5**), as well as the known compounds decarestrictine D (**6**) and sterigmatocystin (**7**), which were identified by comparison of their NMR and MS data with literature values.[5,6] Compounds **1**–**5** were minor constituents, while **6** and sterigmatocystin were relatively abundant. Sterigmatocystin is known to have antiinsectan activity[6] and appears to be responsible for most of the activity of the extract.

The molecular formula of botryolide A (**1**) was determined to be $C_{10}H_{14}O_5$ on the basis of HRESIMS data. The NMR data for **1** (Table 1) suggested that it is a member of the decarestrictine class, a group of 10-membered lactone-containing polyketides possessing a variety of substitution patterns.[5,7] Aside from resonances for an ester carbonyl and a methyl group connected to an oxygenated methine, which are typical of members of this structural class, the ^{1}H and ^{13}C NMR data included signals for two 1,2-disubstituted epoxide units. Since none of the known decarestrictine analogues possess more than one epoxide unit, it was evident that **1** is a new compound.[5,7] COSY data revealed a single continuous spin-system, as is often observed for decarestrictine-type compounds. This spin-system consists of the H_2-2 to H_3-10 system in **1** and enabled location of the epoxide units at adjacent positions within the molecule (C-4–C-5 and C-6–C-7). ^{1}H NMR J values suggested that the epoxides have C-4–C-5 *trans* (J = 2.3 Hz) and C-6–C-7 *cis* (J = 4.6 Hz) configurations and that one of the C-8 methylene protons (δ 1.76) is nearly *anti* to both H-7 and H-9 (vicinal J values of 11.0 and 10.4 Hz). Combining these data with NOESY correlations (Figure 1) suggested the relative configuration shown. However, the flexibility of the molecule required caution in assignment of stereochemical features based on these data alone. Fortunately, crystallization of **1** enabled X-ray diffraction analysis. The NOESY correlations summarized in Figure 1 were fully consistent with the relative configuration obtained from the X-ray crystal structure (Figure 2).

Botryolide B (**2**) was assigned the molecular formula $C_{10}H_{14}O_4$ on the basis of NMR and HRESIMS data. Analysis of ^{1}H NMR (Table 1) and decoupling data for **2** was straightforward and led to assignment of a gross structure that matches those of the known metabolites decarestrictines A_1 and A_2.[5] However, in the case of **2**, the $J_{H4\text{-}H5}$ value (11 Hz) was significantly smaller than that of decarestrictine A_1 or A_2 (15–16 Hz), indicating the presence of a *cis* C-4–C-5 olefin in the 10-membered lactone ring, rather than a *trans* olefin. As in the data for **1**, one of the C-8 methylene protons (δ 1.23 in this instance) is nearly *anti* to both H-7 and H-9 (vicinal J values of 11 Hz). NOESY correlations between the epoxide protons H-6 and H-7 and from oxymethine proton H-9 to H-7 suggested that both epoxide protons are again on the same face of the molecule as H-9, although the $J_{H6\text{-}H7}$ value was reduced slightly relative to that observed for **1**. A strong correlation from epoxide proton H-6 to the oxymethine proton H-3 was also observed. Energy-minimized molecular models (Chem 3D Pro 9.0) indicate that such a correlation is likely only if H-3 is on the same face of the macrocycle as the epoxide protons, thereby leading to proposal of the relative configuration shown in **2**.

Botryolide C (**3**) was assigned the molecular formula $C_{13}H_{16}O_6$ on the basis of HRESIMS and ^{13}C NMR data. The formula deviates from that of decarestrictine-type compounds such as **1** and **2** by the presence of three additional carbons. Even so, the data showed significant resemblance to those of **1** and **2**. Aside from a decarestrictine-like spin-system (Table 1) corresponding to the C-4–C-10 unit of **3**, and an isolated aliphatic methylene unit (H_2-2), compound **3** also contains an isolated $OCHCH_2$ unit (δ_H 2.86 and 2.89/δ_C 38.9 and δ_H 4.56/δ_C 68.9), a second ester or acid carbonyl carbon (δ 175.0), and an oxygenated quaternary sp^3 carbon (δ 85.0). Analysis of HMBC data showed correlations from the methylene protons at δ_H 2.86 and 2.89 (H-11a and H-11b) to C-2, C-3, C-4, C-12, and C-13, while the oxygenated methine proton signal at δ_H 4.56 (H-12, dd, J = 13.6, 6.1) showed correlations to C-3, C-11, and C-13. These data, together with additional HMBC

[⊥] Dedicated to Dr. G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

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Table 1. ^{1}H and ^{13}C NMR Data for Botryolides A (**1**), B (**2**), and D (**4**) in $CDCl_3$[a]

	botryolide A (**1**)		botryolide B (**2**)		botryolide D (**4**)	
C#	δ_H (mult.; *J*)	δ_C	δ_H (mult.; *J*)	δ_C	δ_H (mult.; *J*)	δ_C
1		169.0		170.0		170.0
2α	2.53 (dd, 15, 10)	43.3	2.66 (dd, 11, 3.7)	43.5	2.35 (dd, 16, 11)[c]	43.0
2*β*	3.00 (dd, 15, 6.8)		2.43 (dd, 11, 10)		2.76 (ddd, 16, 3.0, 1.2)[c]	
3	3.67 (ddd, 10, 8.9, 6.8)	72.9	4.88 (m); 1.98 (OH; d, 3.3)[b]	67.8	4.30 (tt, 11, 3.0)	65.0
4	2.89 (dd, 8.9, 2.3)	59.0	5.70 (ddd, 11, 8.2, 1.9)	128.0	1.60 (m); 2.53 (m)	31.1
5	2.76 (dd, 8.2, 2.3)	60.0	5.44 (ddd, 11, 5.6, 1.0)	135.0	2.63 (ddd, 19, 13, 3.6)	34.0
					2.26 (dt, 19, 4.1)	
6	2.63 (dd, 8.2, 4.6)	58.4	3.47 (ddd, 5.6, 3.5, 1.9)	57.0		210.0
7	3.07 (ddd, 10, 4.8, 4.6)	54.8	2.98 (dt, 11, 3.5)	53.5	4.20 (dd, 6.0, 4.6)	74.5
8α	2.32 (br dd, 15, 4.8)	38.8	2.18 (ddd, 14, 3.5, 1.5)	38.0	2.46 (dt, 15, 4.6)	38.6
8*β*	1.76 (ddd, 15, 11, 10)		1.23 (dt, 14, 11)		2.05 (ddd, 15, 11, 6.0)	
9	5.00 (br dq, 11, 6.3)	72.6	5.27 (ddq, 11, 1.5, 6.4)	67.7	5.10 (ddq, 11, 4.2, 6.6)	69.3
10	1.37 (d, 6.3)	23.8	1.28 (d, 6.4)	20.5	1.30 (d, 6.6)	20.0

[a] ^{1}H NMR data for **1** and **4** were recorded at 600 MHz. Those for **2** were recorded at 400 MHz. All ^{13}C NMR data were recorded at 75 MHz. [b] This signal appeared only in a ^{1}H NMR spectrum recorded at 300 MHz. [c] These assignments may be interchanged.

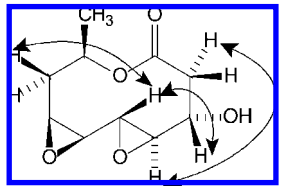


Figure 1. Key NOESY correlations for botryolide A (**1**).

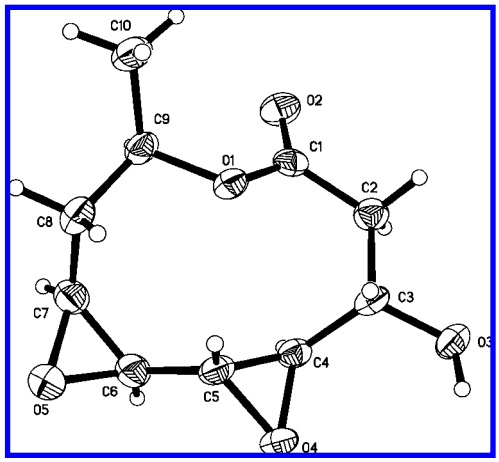


Figure 2. X-ray model of botryolide A (**1**).

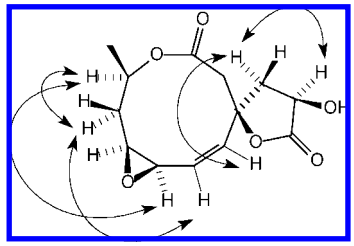


Figure 3. Selected NOESY correlations for botryolide C (**3**).

correlations consistent with the other subunits noted above, suggested the presence of a five-membered ring spiro-fused to the 10-membered lactone at quaternary oxygenated carbon C-3. NOESY correlations (Figure 3) suggested the relative configuration shown in **3**. However, the olefin geometry in **3** could not be definitively assigned on the basis of NMR data, as its coupling constant (J = 13.1) lies at the boundary between *cis* and *trans J* values. An X-ray diffraction study conducted using crystals of **3** obtained from EtOAc−$CHCl_3$ confirmed the proposed structure and relative configuration and unambiguously established that the C-4–C-5

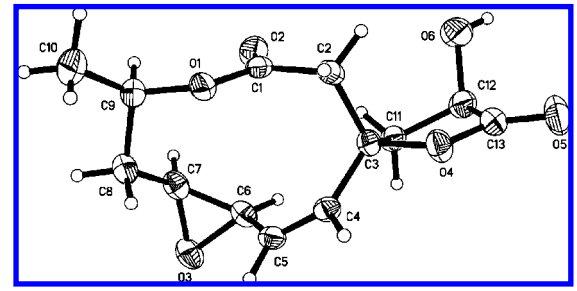
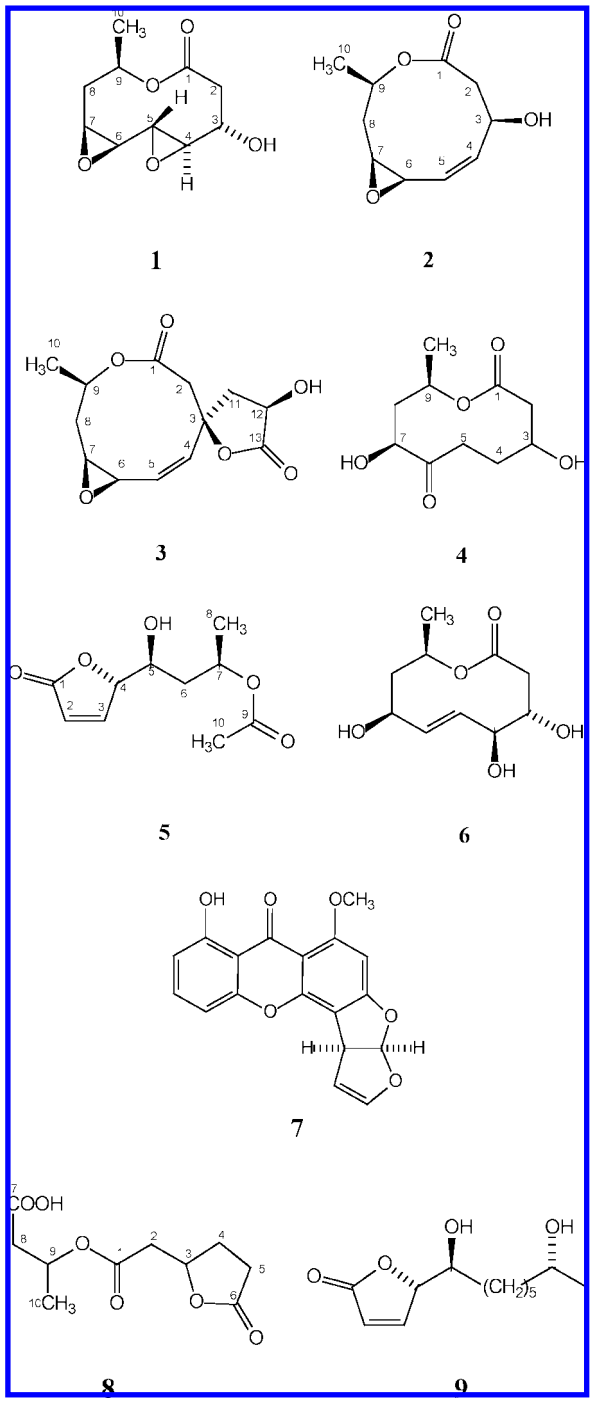


Figure 4. X-ray model of botryolide C (**3**).

double bond has the *Z* configuration. The presence of the three-carbon unit corresponding to C-11–C-13 in **3** appears to be unprecedented among members of this class.

Botryolide D (**4**) has the molecular formula $C_{10}H_{16}O_5$, as determined by analysis of its HRESIMS data. The ^{13}C NMR spectrum of **4** included a ketone carbonyl signal at δ_C 210.0 in addition to the ester carbonyl signal at δ_C 170.0. To our knowledge, only two decarestrictine analogues with this molecular formula that incorporate ketone units have been previously reported (decarestrictine G and cephalosporolide C),[7,8] and the NMR data for **4** do not match the data for either of these compounds. COSY data revealed the presence of two isolated spin-systems corresponding to the H_2-2 to H_2-5 and H-7 to H_3-10 units in **4**. HMBC data showed correlations from H_2-8 to C-6, C-7, C-9, and C-10; from H-7 to C-6, C8, and C-9; and from H_2-5 to C-3, C-4, and C-6, thereby locating the ketone moiety between C-5 and C-7. Additional HMBC correlations were fully consistent with assignment of structure **4** to this compound, making it the first member of this class to contain a ketone group at C-6.

Although the utility of NOESY data for stereochemical analysis of similar structures was demonstrated by the agreement between NOESY-based conclusions and the X-ray data for **1** and **3** above, compound **4** is conformationally more flexible due to the smaller number of sp^2 carbons and the absence of other geometrical constraints in the structure. As a result, the relative configuration of **4** was more difficult to assign on the basis of NOESY and ^{1}H NMR *J* values, particularly for C-3. While the data were consistent with having the configurations at C-7 and C-9 match those of the other metabolites, that of C-3 could not be assigned with confidence. Further efforts to assign the relative configuration for botryolide D (**4**) were precluded by gradual decomposition. A product of this process was obtained in submilligram amounts and identified by analysis of MS and NMR data. HRESIMS indicated a molecular formula of $C_{10}H_{14}O_6$ based on HRESIMS data. Analysis of HMBC data led to straightforward assignment of gross structure **8** for this compound, which is a product of oxidative cleavage at the C-6–C-7 bond of **4**, followed by lactonization. Although this spontaneous



decomposition was considered somewhat surprising, the vicinal oxidation pattern at C-6 and C-7 (not present in any of the other metabolites) is presumably a factor in making **4** susceptible to autoxidation.

Botryolide E (**5**) was assigned the molecular formula $C_{10}H_{14}O_5$ on the basis of its NMR and ESIMS data. Once again, a single spin-system (corresponding to the C-2–C-8 unit in **5**) could be ascertained by inspection of the ^{1}H NMR data. However, in this instance, the NMR spectra revealed some significant differences relative to the decarestrictine-type compounds. The coupling constants and δ values of the olefinic proton signals at δ_H 6.18 (dd, J = 5.7, 1.8, H-2) and δ_H 7.47 (dd, J = 5.7, 1.8, H-3) are suggestive of the double bond in an α,β-unsaturated-γ-lactone unit.[9–11] An acetate unit was also present (δ_H 2.01, s/δ_C 21.2, 172.0), and oxygenated methine proton H-7 (δ 5.09, m) showed a correlation to the acetate carbonyl, leading to assignment of gross structure **5** for botryolide E.

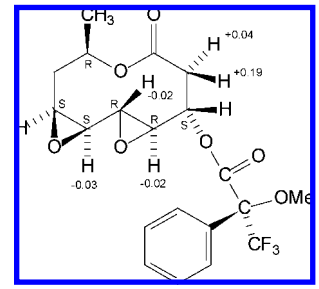


Figure 5. $\Delta\delta$ values ($\delta_S - \delta_R$) for the MTPA esters of botryolide A (**1**).

Table 2. ^{1}H and ^{13}C NMR Data for Botryolide C (**3**) in $CDCl_3$

C#	δ_H (mult.; J)	δ_C
1		168.2
2	2.83 (d, 11), 2.85 (d, 11)	45.8
3		85.0
4	5.86 (dd, 13, 2.0)	133.9
5	5.50 (dd, 13, 5.5)	129.1
6	3.53 (ddd, 5.5, 3.7, 2.0)	52.4
7	3.08 (dt, 11, 3.7)	57.5
8	2.25 (ddd, 14, 3.7, 1.1)	38.0
	1.25 (ddd, 14, 11, 11)	
9	5.38 (ddq, 11, 1.1, 6.5)	68.1
10	1.32 (d, 6.5)	20.5
11	2.86 (dd, 7.4, 14)	38.9
	2.89 (dd, 14, 6.3)	
12	4.56 (dd, 7.4, 6.3)	68.9
13		175.0

The relative configuration at C-4 and C-5 of **5** was assigned by NMR comparison with *erythro* and *threo* isomers of the model compound *iso*-cladospolide B.[9] The chemical shifts and coupling constants of H-2, H-3, and H-4 in **5** matched more closely with the corresponding signals for the *threo* isomer of *iso*-cladospolide B (**9**) than the *erythro* isomer. The multiplicity of the H-4 signal for *threo* isomer **9** was reported as a quintet (J_H =1.8 Hz) in the literature. Although the H-4 signal in **5** is also an apparent quintet at 300 MHz, analysis at 600 MHz revealed that the signal is actually a doublet of triplets with 3.2- and 1.8-Hz coupling constants. The relative configuration at C-7 with respect to C-5 was presumed to be as shown by analogy to that of related compounds **1**–**4**.

The absolute configuration of botryolide A (**1**) was assigned using the modified Mosher method.[12] NMR comparison of the *R*- and *S*-MTPA esters of **1** revealed that the most significant $\Delta\delta$ differences between the *R*- and *S*-derivatives were observed for the methylene signals at C-2; however, all of the observed data (Figure 5) were consistent with assignment of the 3*S*, 4*R*, 5*R*, 6*S*, 7*S*, 9*R* configuration for **1**. In view of the presumed biosynthetic analogies with **1**, the absolute configurations for **2**–**4** are proposed to be analogous to that of **1**, as shown. The previously known decarestrictines for which assignments have been made have all been proposed to have the same absolute configuration as botryolide A at the C-9 position.[5,16–18] In addition, the configuration at C-3 of botryolides A (**1**) and D (**4**) matches that of decarestrictines A_2, C_1, and D, while the C-7 configuration of **4** matches that of the co-occurring decarestrictine D (**6**). The C-6–C-7 epoxide configuration in **1**, **2**, and **3** matches that reported for decarestrictines A and B. All of these data suggest a stereochemically similar polyketide biosynthetic pathway for the botryolides and the decarestrictines. Compound **5** may be a biosynthetic shunt product of the pathway in which only four acetate units are condensed to form the polyketide chain, and a further acetate unit is added by acylation of the oxygen atom at C-7. If this is the case, C-7 of **5** would presumably have the *R*-absolute configuration by analogy to the C-9 positions of botryolides A−D. Finally, the unusual three-carbon unit appended to the polyketide chain in **3** (i.e., correspond-

ing to C-11–C-13) seems most likely to arise from condensation of the pentaketide unit with a four-carbon precursor derived from the TCA cycle, accompanied by decarboxylation. Similar three-carbon units occurring in other types of fungal metabolites such as canescin and marticin have been shown to arise from such processes.[19,20]

As noted above, botryolides A−E are closely related to decarestrictines,[7] a series of polyketide-derived metabolites originally reported from several *Penicillium* isolates, including *P. simplicissimum*. Like botryolides A−D (**1**−**4**), most of the decarestrictines contain 10-membered ring lactone (decanolide) units. Other decanolide derivatives have also been described as fungal metabolites, and those with similar pentaketide origins include cephalosporolides,[8] aspinolides,[13] diplodialides,[14] humicolactone,[15] pyrenolides,[16] multiplolide,[17] and modiolides.[18] Some of the known decarestrictines are inhibitors of cholesterol biosynthesis,[7] but a wide range of other bioactivities have also been described for members of this general class.

Botryolides A−D (**1**–**4**) did not show activity in standard disk assays against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6051), or *Candida albicans* (ATCC 14053) at 100 μg/disk. Botryolides A, B, and D were also inactive against *Aspergillus flavus* (NRRL 6541) and *Fusarium verticillioides* (NRRL 25457) at the same test level. Botryolides C and E were not tested due to sample limitations. As noted earlier, the insect activity of the original extract was attributed to significant quantities of sterigmatocystin (**7**) found to be present.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Rudolph automatic polarimeter, model APIII. Melting points were obtained using a Fisher-Johns micromelting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC-300, DRX-400, and AMX-600 instruments, while HMQC, HMBC, COSY, and NOESY data were recorded on a Bruker AMX-600. HPLC was carried out using a Beckman System Gold HPLC system with a model 168 UV detector and an Alltech Hyperprep BDS 8-μm C_{18} column (4.6 × 250 mm) at a flow rate of 2 mL/min with UV detection at 216 nm.

Isolation, Cultivation, and Fermentation of Fungal Material. The isolate of the *Botryotrichum* state of *Chaetomium piluliferum* Daniels (MYC-1117) was obtained from the surface of an unidentified polypore on a dead hardwood branch of live oak collected in a palmetto habitat at Alligator Point, Panacea, FL, on April 30, 2000. A subculture of this isolate was deposited at the USDA NRRL culture collection at the National Center for Agricultural Utilization Research, Peoria, IL, under accession number NRRL 38180. The fungal isolate was grown on 100 g of rice for 30 days at 25 °C and extracted with EtOAc using general protocols described previously.[1]

Extraction and Isolation. The crude extract (2.7 g) was partitioned between hexanes and MeCN. The MeCN fraction (2.5 g) was fractionated on a silica gel column using a hexanes−EtOAc−MeOH gradient to afford 19 fractions. Fractions 2–6 (424 mg) were comprised mostly of sterigmatocystin (**7**), with fraction 3 consisting of a sample of pure material (43 mg). Fraction 9 (53 mg) eluted with 20% hexane in EtOAc was further separated by reversed-phase HPLC (20% MeCN−H_2O for 10 min, 20–30% over 5 min, 30% for 10 min, 30–40% over 2 min, 40% for 10 min, 40–50% over 5 min, 50–100% over 2 min) to afford botryolide A (**1**; 1.5 mg), botryolide B (**2**; 7 mg), and botryolide C (**3**; 2 mg). Fraction 10 (163 mg) was separated by reversed-phase HPLC (20% MeCN−H_2O for 15 min, 20–30% over 10 min, 30% for 10 min, 30–40% over 2 min, 40–100% over 5 min) to afford another sample of botryolide A (**1**; 4 mg) and a fraction (47 mg) that, upon further reversed-phase HPLC (10–30% MeCN−H_2O for 20 min, 30–100% over 10 min), yielded botryolide E (**5**; 1 mg). Fraction 14 (82 mg) was separated by reversed-phase HPLC (10–40% MeCN−H_2O over 30 min, 40–60% over 5 min, 60–100% over 5 min) to afford botryolide D (**4**; 8 mg). Fraction 16 (100 mg) was separated by reversed-phase HPLC (10–30% MeCN−H_2O over 30 min, 30–60% over 5 min, 60–100% over 5 min) to afford the known compound decarestrictine D (**6**; 16 mg).

Botryolide A (1): colorless crystals ($CHCl_3$−EtOAc); mp 125–126 °C; $[\alpha]^{25}_D$ −9.3 (*c* 0.14, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 1; HMBC data, H-2 → C-1, 4, 3; H-3 → C-2, 4; H-4 → C-2, 6, 5, 9; H-5 → C-3, 4, 6; H-6 → C-5, 7; H-7 → C-7, 8; H-8 → C-6, 7, 10; H-9 → C-1, 7, 8, 10; H-10 → C-7, 8, 9; HRESIMS *m/z* 215.0925 $[M + H]^+$, calcd for $C_{10}H_{15}O_5$, 215.0915.

Botryolide B (2): colorless glass; $[\alpha]^{25}_D$ –144 (*c* 0.23, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 199.0964 $[M + H]^+$, calcd for $C_{10}H_{15}O_4$, 199.0966.

Botryolide C (3): colorless crystals ($CHCl_3$−EtOAc); mp 163–165 °C; $[\alpha]^{25}_D$ −8.7 (*c* 0.15, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 2; HMBC data, H-2 → C-1, 3, 4, 11; H-4 → C-2, 5, 6, 11; H-5 → C-3, 4, 6, 7; H-6 → C-4, 5, 7; H-7 → C-6, 8; H-8 → C-6, 7; H-9 → C-7, 8, 10; H-10 → C-8, 9; HRESIMS *m/z* 291.0842 $[M + Na]^+$, calcd for $C_{13}H_{16}O_6Na$, 291.0840.

Botryolide D (4): colorless glass; $[\alpha]^{25}_D$ –2.1 (*c* 0.4, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 1; HMBC data, H-2 → C-1, 3, 4; H-3 → C-1, 2, 4, 5; H-4 → C-2, 3, 5, 6; H-5→ C-3, 4, 6; H-7 → C-6, 8, 9; H-8 → C-6, 7, 9, 10; H-9 → C-1, 7, 10; H-10 → C-8, 9; HRESIMS *m/z* 217.1045 $[M + H]^+$, calcd for $C_{10}H_{17}O_5$, 217.1075.

Botryolide E (5): colorless glass; $[\alpha]^{25}_D$ −38 (*c* 0.05, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (dd, J = 5.7, 1.8, H-3), 6.18 (dd, J = 5.7, 1.8, H-2), 5.09 (ddq, J = 9.8, 6.3, 3.3, H-7), 5.01 (dt, J = 3.2, 1.8, H-4), 3.89 (dt, J = 11, 3.2, H-5), 2.01 (s, H-9), 1.80 (ddd, J = 14, 9.8, 3.2, H-8a), 1.73 (ddd, J = 14, 11, 3.3, H-8b), 1.25 (d, J = 6.3, 10); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9 (C-1), 172.0 (C-9), 153.4 (C-2), 123.0 (C-3), 85.0 (C-4), 67.6 (C-5), 67.4 (C-7), 39.1 (C-6), 21.2 (C-10), 20.7 (C-8); HMBC data, H-2 → C-1, 3, 4; H-3 → C-1, 2; H-4 → C-2, 3, 5; H-5 → C-2, 4, 6; H-6 → C-5, 7, 10; H-7 → C-5, 6, 8, 10; H-9 → C-8; H-10 → 6, 7; ESIMS *m/z* 215 ($[M + H]^+$; 9), 237 ($[M + Na]^+$; 23), 451 ($[2M + Na]^+$; 100); HRESIMS *m/z* 237.0739 ($[M + Na]^+$), calcd for $C_{10}H_{14}O_5Na$, 237.0739.

Oxidation Product (8) of Botryolide D: colorless glass; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.32 (dd, J = 14, 6.5, H-9), 4.86 (pentet, J = 6.6, H-3), 2.76 (dd, J = 16, 6.6, Ha-2), 2.68 (dd, J = 16, 8.0, Ha-8), 2.60 (dd, J = 16, 6.6, Hb-2), 2.58 (m, H-5a), 2.56 (m, H-5b), 2.54, (m, H-8b), 2.44 (m, Ha-4), 1.96 (m, Hb-4), 1.33 (d, J = 6.5, H_3-10); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8 (C-1), 40.2 (C-2), 76.3 (C-3), 27.4 (C-4), 28.7 (C-5), 176.8 (C-6), 171.4 (C-7), 40.0 (C-8), 68.0 (C-9), 20.0 (C-10); HMBC data, H_2-2 → C-1, C-3, C-4; H-3 → C-1; H_2-4 → C-2, C-3, C-5, C-6; H_2-5 → C-6; H_2-8 → C-7, C-9; H-9 → C-1; H_3-10 → C-8, C-9; HRESIMS *m/z* 253.0675 $[M + Na]^+$, calcd for $C_{10}H_{14}O_6Na$, 253.0688.

X-ray Crystallographic Analysis of Botryolide A (1)..[21] A colorless needle of **1** (0.34 × 0.03 × 0.03 mm) was obtained from $CHCl_3$−EtOAc, and had cell dimensions a = 16.0871(16) Å, b = 16.0871(16) Å, c = 7.4888(7) Å. Data for **1** were collected with a Nonius KappaCCD diffractometer (Mo Kα radiation, graphite monochromator) at 190(2) K (cold N_2 gas stream) using standard CCD techniques, yielding 19 643 data. Lorentz and polarization corrections were applied. A correction for absorption using the multiscan technique was applied (T_{max} = 0.997, T_{min} = 0.964). Equivalent data were averaged yielding 1076 unique data (R_{int} = 0.073, 899$F > 4\sigma(F)$, Friedel pairs averaged). On the basis of a preliminary examination of the crystal, the space group $P6_1$ was assigned (no exceptions to the systematic absence 001, 1 = Mod(6) + 1, 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 illustrations are at the 50% level.

All non-hydrogen atoms were refined with anisotropic thermal parameters. All H atoms were included with the riding model using the XL program default values. A disordered solvent molecule of EtOAc was present in the structure oriented along the 6_1 axis. It was refined as a rigid group with occupancy = 1/6 and a single isotropic thermal parameter for C and O atoms. All H atoms were added with the riding atom model. No further restraints or constraints were imposed on the refinement model. The final R indices gave R_1 = 0.0420, wR_2 = 0.1048.

X-ray Crystallographic Analysis of Botryolide C (3)..[21] A colorless rod of **3** (0.38 × 0.10 × 0.08 mm), obtained from $CHCl_3$−EtOAc, proved to be orthorhombic with cell dimensions a = 5.7299(6) Å, b = 11.7665(12) Å, c = 18.8714(19) Å. Data were

collected using standard CCD techniques as described above for **1** yielding 20 000 data. Lorentz and polarization corrections were applied ($T_{max} = 0.99$, $T_{min} = 0.96$). Equivalent data were averaged yielding 1716 unique data ($R_{int} = 0.035$, $1494F > 4\sigma(F)$, Friedel pairs averaged). On the basis of a preliminary examination of the crystal, the space group $P2_12_12_1$ was assigned (no exceptions to the systematic absences H00, H = odd, 00L, L = odd, were noted). The same computer programs employed for analysis of **1** were again used for data reduction, refinement, tabulation, and illustration. No further restraints or constraints were imposed on the refinement model. The final R indices gave $R_1 = 0.0321$, $wR_2 = 0.0693$.

Preparation of (*R*) and (*S*)-MTPA Esters of 1. A solution of **1** (2.3 mg, 0.011 mmol) in pyridine (300 μL) was treated with (*R*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPACl; 20 μL), and the mixture was stirred at 25 °C for 50 h. Aqueous saturated $NaHCO_3$ (2 mL) was added, and the solution was extracted with CH_2Cl_2 (3 × 3.0 mL). The combined organic extracts was evaporated to dryness and subjected to RP-HPLC (Alltech HS BDS 10-μm C_{18} column, 250 × 10 mm, flow rate 2 mL/min, UV detection at 242 nm, eluted with MeCN−H_2O, 20–100% over 40 min) to afford the (*S*)-MTPA ester of **1** (1 mg). Analogous treatment of another sample of **1** (3.0 mg, 0.014 mmol) with (*S*)-MTPACl afforded the (*R*)-MTPA ester of **1** (0.7 mg). ^{1}H NMR data for the (*R*)-MTPA ester of **1** (600 MHz, $CDCl_3$): δ 5.27, (ddq, J = 10, 3.0, 6.3, H-9), 4.97 (ddd, J = 11, 9.3, 6.0, H-3), 4.28 (dd, J = 9.3, 4.2, H-4), 3.49 (dd, J = 8.5, 4.2, H-5), 3.26 (dd, J = 8.5, 2.2, H-6), 3.20 (dd, J = 9.0, 2.2, H-7), 3.07 (dd, J = 18, 6.0, H-2a), 2.59 (dd, J = 18, 11, H-2b), 2.39 (ddd, J = 16, 10, 0.6, H-8a), 2.18 (ddd, J = 16, 9.0, 3.0, H-8b), 1.35 (d, J = 6.3, H_3-10). ^{1}H NMR data for the (*S*)-MTPA ester of **1** (600 MHz, $CDCl_3$): δ 5.29, (ddq, J = 10, 3.0, 6.3, H-9), 5.01 (ddd, J = 11, 9.0, 6.0, H-3), 4.28 (dd, J = 9.0, 4.7, H-4), 3.46 (dd, J = 8.2, 4.7, H-5), 3.24 (dd, J = 8.2, 2.0, H-6), 3.18 (ddd, J = 8.7, 2.0, 1.2, H-7), 3.11 (dd, J = 17, 6.0, H-2a), 2.78 (dd, J = 17, 11, H-2b), 2.40 (ddd, J = 16, 10, 1.2, H-8a), 2.16 (ddd, J = 16, 8.7, 3.0, H-8b), 1.35 (d, J = 6.3, H_3-10).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of botryolides A−C (**1**−**3**). This material is available free of charge on the Internet at http://pubs.acs.org.

References and Notes

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(21) Crystallographic data for compounds **1** and **3** have been deposited with the Cambridge Crystallographic Data Center (deposition numbers CCDC 609824 for **1** and 609825 for **3**). The 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 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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