Botryane Metabolites from the Fungus *Geniculosporium* sp. Isolated from the Marine Red Alga *Polysiphonia*

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Received May 27, 2004

Eleven new botryane metabolites (**1**−**11**) were isolated together with four known cytochalasins (**12**−**15**) from the mitosporic fungus *Geniculosporium* sp., which is associated with the red alga *Polysiphonia* sp. The structures of **1**−**11** differ from known botryanes in substitution pattern, degree of saturation, and altered sites of oxidation, alkylation, unsaturation, etc. They were determined by spectroscopic methods (mainly extensive 1D and 2D NMR experiments and mass spectral measurements) and X-ray single-crystal analysis. The herbicidal, antifungal, and antibacterial activities of these new natural products were evaluated.

In the search for new pharmaceutical or agrochemical lead structures, much attention is being paid to secondary metabolites from marine microorganisms, including marine fungi.[1,2] In connection with our ongoing search for biologically active metabolites from fungi, we investigated the constituents of *Geniculosporium* sp., internal strain number 6580, a fungus that is associated with the red alga *Polysiphonia* sp., from the Baltic Sea at Ahrenshoop, Germany. From this fungus, we isolated 11 new tricyclic sesquiterpenes with the botryane skeleton, related to those isolated by Collado et al. from *Botrytis cinerea*.[3−7] The new botrydial sesquiterpenoids are 7-hydroxy-10-methoxydeacetyldihydrobotrydial (**1a**), 7-hydroxy-10-oxodehydrodihydrobotrydial (**2**), 7,10-dihydroxydehydrodihydrobotrydial (**3**), 7-hydroxy-10-methoxydehydrodihydrobotrydial (**4**), 7-hydroxy-10-ethoxydehydrodihydrobotrydial (**5**), 7-hydroxy-10-dehydroxydehydrodihydrobotrydial (**6**), 7-hydroxydeacetylbotryenalol (**7**), 7,10-dihydroxydeacetyldihydrobotrydial-1(10)-ene (**8**), 4,10-didehydroxy-7-hydroxydeacetyldihydrobotrydial-1(10),5(9)-diene (**9**), 7-hydroxy-10-dehydroxydeacetyldihydrobotrydial-1(10),5(9)-diene (**10**), and 15α-hydroxy-14-aldehyde probotryan-4(5)-ene (**11**). In addition, four known cytochalasins, L-696,474 (**12**),[8] cytochalasin U (**13**),[8] RKS-1778 (**14**),[9] and cytochalasin H (**15**)[10] (Chart 1), were also isolated from the ethyl acetate extract. This appears to be the first reported occurrence of botryane metabolites in the genus *Geniculosporium*. Here we describe the isolation and structural elucidation of these new compounds and their antimicrobial activity.

Results and Discussion

Compound **1a** was obtained as colorless crystals with the molecular formula $C_{16}H_{28}O_5$ as deduced from mass spectral and C NMR spectroscopic data. Its IR spectrum showed strong absorptions for hydroxyl groups (3500, 3420 cm^{-1}), while the ^1H NMR spectrum (Table 1) exhibited the presence of five methyl groups, of which four were singlets (δ 1.13, 1.19, 1.29, 3.39) and one was a doublet (δ 0.99, d, J = 6.3 Hz), and five protons on oxygenated carbons at δ 3.96 (s), 3.88 (td, J = 10.1, 4.8 Hz), 4.85 (s), 4.04 (d, J = 10.4 Hz), and 3.24 (d, J = 10.4 Hz), respectively. The ^{13}C NMR spectrum of **1a** (Table 3) showed signals for 16 carbons, and the DEPT spectra indicated the presence of five methyls, two methylenes, six methines, and three quaternary carbon atoms. The spectroscopic data of this compound were similar to those of deacetyldihydrobotrydial (**1c**), a sesquiterpene with the botrydial skeleton previously isolated from *Botrytis cinerea*, for which the stereochemistry has already been established.[3,4,11] The main differences in the ^1H NMR spectra of the two compounds were that a methoxy group was added at C-10 and a hydroxyl group at C-7. These changes caused the downfield shifts of H-7 and H-10 in compound **1a**. Comparison of the ^{13}C NMR spectroscopic data of compound **1a** with those of deacetyldihydrobotrydial showed the analogy of the chemical shifts, except for C-7 and C-10. This suggested that compound **1a** was a botrydial sesquiterpene with a hydroxyl group located on C-7 and a methoxy group on C-10.

Treatment of **1a** with pyridine and acetic anhydride gave diacetate **1b**. The shifts observed for the signals of C-4 and C-7 in **1b** from δ 68.9 to 72.1 and δ 82.0 to 84.6 with respect to those in **1a** were consistent with the proposed structure in which two secondary hydroxyl groups were located on C-4 and C-7 in **1a**. Analysis of the COSY and HMQC spectra of **1a** enabled the deduction of the fragment −CH(10)−CH(1)−CH(2)(CH_3(11))−CH_2(3)−CH(4)−CH(5)−. In the HMBC spectrum of **1a**, $^{13}C-^1H$ long-range correlations were found between C-6 and H-4, H-5, H-7, H-12, H-13; C-8 and H-5, H-7, H-14, H-15; C-9 and H-1, H-2, H-5, H-14, H-15; and C-10 and H-1, H-15, 10-OCH_3 (Figure 1). The NOESY spectrum exhibited clear correlations between H-7 and H-5, H-15β and between H-5 and H-3β, showing that these protons are on the same side of the molecule. Therefore, compound **1a** was assigned as 7-hydroxy-10-methoxydeacetyldihydrobotrydial**.** The NMR spectral data of deacetyldihydrobotrydial (**1c**) are listed in Tables 1 and 3 for comparison.

The assignment and stereochemistry of compound **1a** were confirmed unambiguously by X-ray diffraction analysis of a single crystal obtained from methanol (Figure 2). The absolute configuration has not yet been elucidated. However, we suggest that these compounds and the botryanes have the same absolute configuration because the optical activity of most representatives is positive,[3] although deviating in magnitude.

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10.1021/np0498206 CCC: $30.25
Published on Web 03/05/2005

Chart 1

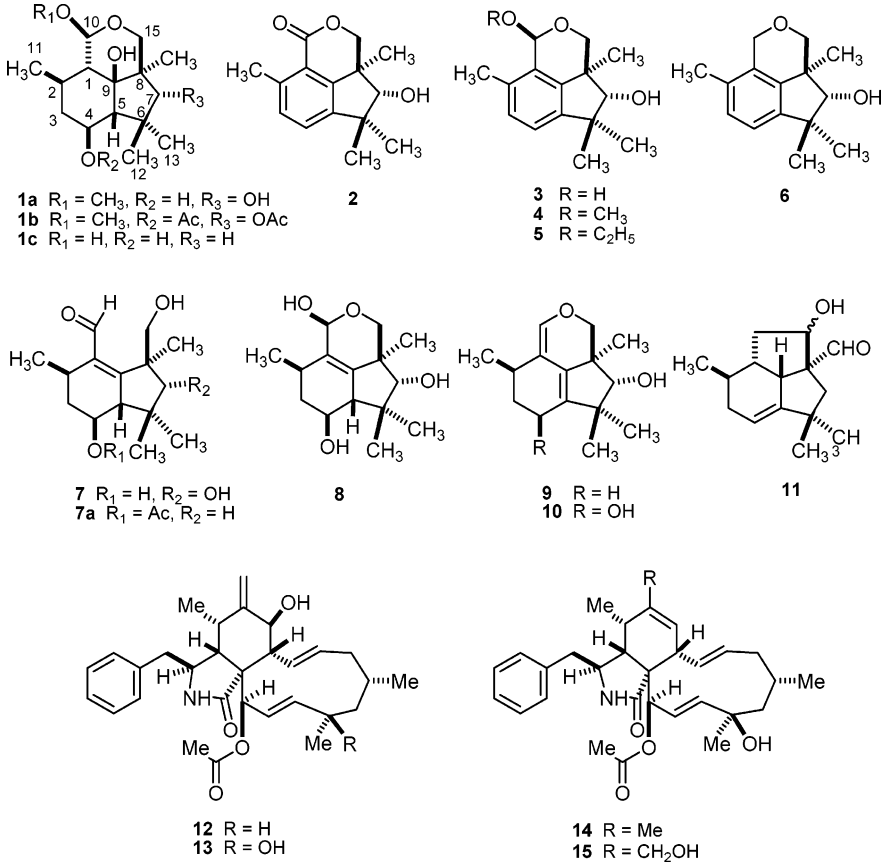

Table 1. ^{1}H NMR Spectral Data of Compounds **1**–**5**[a] ($CDCl_3$, **1a** in CD_3OD)

proton	**1a**[b]	**1b**[c]	**1c**[e]	**2**[b]	**3**[d]	**4**[d]	**5**[d]
1	1.47 (d, 12.4)	1.51 (d, 12.5)	2.03 (d, 12.2)				
2	1.78 m	1.80 m	1.85 (ddd, 12.2, 12.0, 2.7)				
3α	1.89 m	1.97 m	2.16 (ddd, 12.5, 4.6, 2.7)	7.20 (d, 7.7)	7.04 (d, 8.0)	7.04 (d, 8.0)	7.03 (d, 8.0)
3*β*	1.10 m	1.00 m	1.44 (dd, 12.5, 12.0)				
4	3.88 (td, 4.8, 10.1)	5.06 (td, 4.8, 10.6)	4.21 (dd, 4.6, 9.8)	7.29 (d, 7.7)	7.08 (d, 8.0)	7.07 (d, 8.0)	7.08 (d, 8.0)
5	1.61 (d, 10.1)	1.93 (d, 10.6)	2.25 (d, 9.8)				
7	3.96 s	4.97 s	1.24 (d, 11.2)	3.90 s	3.55 s	3.58 s	3.80 s
10	4.85 s	4.79 s	5.74 s		5.28 s	5.35 s	5.69 s
11	0.99 (d, 6.3)	0.93 (d, 6.0)	0.96 (d, 6.1)	2.60 s	2.32 s	2.30 s	2.32 s
12	1.19 s	0.94 s	1.63 s	1.36 s	1.35 s	1.35 s	1.35 s
13	1.29 s	1.25 s	1.67 s	1.32 s	1.28 s	1.29 s	1.31 s
14	1.13 s	1.13 s	1.42 s	1.46 s	1.39 s	1.39 s	1.39 s
15α	3.24 (d, 10.4)	3.14 (d, 11.1)	3.39 (d, 10.3)	4.15 (d, 10.1)	3.72 (d, 10.1)	3.55 (d, 10.0)	3.73 (d, 10.0)
15*β*	4.04 (d, 10.4)	4.08 (d, 11.1)	3.46 (d, 10.3)	4.50 (d, 10.1)	3.83 (d, 10.1)	3.80 (d, 10.0)	3.84 (d, 10.0)
OCH_3	3.39 s	3.34 s				3.56 s	
CH_3CO		2.06 s					
		2.03 s					
OCH_2CH_3							4.05 q (5.0)
OCH_2CH_3							1.29 (t, 5.0)

[a] Chemical shift values are in ppm from TMS, and *J* values (in Hz) are presented in parentheses. [b]500 MHz. [c]300 MHz. [d]200 MHz. [e] From T. Kimata et al.[6]

Compound **2** has the molecular formula $C_{15}H_{18}O_3$, as deduced from HREIMS and ^{13}C NMR data. Comparison of the NMR spectrum of **2** (Tables 1, 3) with those of **1a** revealed that **2** possesses a related structure. However, the presence of six aromatic carbon signals in the ^{13}C NMR spectrum of compound **2**, in addition to the downfield chemical shift of the methyl group on C-2 at δ 2.60 ppm (s) in the ^{1}H NMR spectrum, indicated that **2** was an aromatized analogue of **1a**. The absence of signals characteristic of H-10 in the ^{1}H NMR spectrum, together with the IR absorption band at 1700 cm^{-1} for a lactone group and the presence of ^{13}C NMR signals at δ 64.0, was consistent with the proposed structure in which the lactone carbonyl group is located at C-10. The location of the group was further confirmed by the signals for H-15*β* and H-11 correlating to the signal for C-10 (δ 164.0) in the HMBC spectrum of **2** (Figure 1). Comparison of the ^{13}C NMR spectroscopic data with those of 10-oxodehydrodihydrobotrydial,[5] another sesquiterpene with the botrydial skeleton isolated from *Botrytis cinerea*, showed the analogy of the

Table 2. ^{1}H NMR Spectral Data of Compounds **6**−**11**[a] ($CDCl_3$, **7** in CD_3OD)

proton	**6**[d]	**7**[c]	**7a**[e]	**8**[b]	**9**[d]	**10**[c]	**11**[d]
1							2.15 m
2		2.84 m	2.94 m	2.65 m	2.45 m	2.50 m	1.99 m
3α	7.01 (d, 7.8)	2.07 m	2.03 m	2.25 m	1.80 m	1.95 m	2.25 m
3*β*		1.40 m	1.36 m	1.45 m	1.45 m	1.46 m	2.75 (dd, 4.0, 16.0)
4	7.07 (d, 7.8)	3.64 m	4.90 m	3.69 m	2.05 m	3.78 m	5.15 s
5		2.20 (dd, 3.2, 8.9)	2.50 (dd, 3.1, 8.2)	2.10 (dd, 12.1, 4.2)			
7	3.73 s	3.66 s	1.44 (d, 12.9), 1.91 (d, 12.9)	3.60 s	3.57 s	3.48 s	1.67 brs
9							2.10 overlap
10α	4.61 (d, 15.8)	10.27 s	10.18 s	5.22 s	6.15 s	6.22 s	1.68 m
10*β*	4.94 (d, 15.8)						2.45 m
11	2.17 s	1.10 (d, 6.7)	1.05 (d, 6.8)	0.90 (d, 7.2)	1.12 (d, 6.1)	1.00 (d, 6.5)	1.00 (d, 6.3)
12	1.37 s	0.86 s	0.90 s	1.17 s	1.10 s	1.11 s	1.09 s
13	1.32 s	1.29 s	1.12 s	1.31 s	1.25 s	1.05 s	1.20 s
14	1.41 s	1.27 s	1.44 s	1.24 s	1.10 s	1.10 s	9.77 s
15α	3.26 (d, 9.8)	3.40 (d, 10.0)	3.59 (d, 10.5)	3.46 (d, 9.1)	3.43 (d, 9.8)	3.50 (d, 9.9)	3.75 (dd, 7.4, 10.1)
15*β*	4.09 (d, 9.8)	3.58 (d, 10.0)	3.67 (d, 10.5)	3.95 (d, 9.1)	4.09 (d, 9.8)	4.11 (d, 9.9)	

[a] Chemical shift values are in ppm from TMS, and *J* values (in Hz) are presented in parentheses. [b] 500 MHz. [c] 300 MHz. [d] 200 MHz. [e] From I. G. Collado et al.[8]

Table 3. ^{13}C NMR Spectral Data of Compounds **1**−**11**[a]

carbon	**1a**[b]	**1b**[c]	**1c**[e]	**2**[b]	**3**[d]	**4**[d]	**5**[d]	**6**[d]	**7**[c]	**7a**[f]	**8**[b]	**9**[d]	**10**[c]	**11**[d]
1	55.4	54.3	57.3	120.0	134.5	134.5	134.5	129.6	139.5	131.1	130.3	115.5	114.6	42.0
2	28.7	28.0	30.1	140.3	130.2	130.1	130.2	131.8	31.6	29.1	31.8	29.0	24.7	34.9
3	44.3	39.3	46.0	131.9	130.7	130.7	130.6	130.6	42.9	36.6	44.3	33.2	37.0	50.3
4	68.9	72.1	70.3	127.9	123.3	123.4	123.4	121.2	69.0	71.7	69.3	20.5	71.6	136.1
5	62.7	58.4	64.9	144.0	144.6	144.7	144.7	145.3	58.9	58.3	54.7	130.6	133.5	151.3
6	41.2	40.9	39.8	47.4	46.0	46.0	46.0	45.7	44.3	39.1	42.3	42.5	42.3	50.6
7	82.0	84.6	51.8	85.0	85.8	85.8	85.8	85.9	82.9	54.1	84.1	85.4	85.5	54.0
8	48.0	47.9	46.5	43.7	44.1	44.1	44.1	44.0	50.1	51.8	43.6	47.6	47.0	58.1
9	79.3	79.3	84.7	148.0	142.0	141.9	142.1	140.9	167.7	162.8	143.6	135.8	135.5	30.7
10	99.1	98.2	93.3	164.0	92.3	96.1	94.9	65.0	195.9	192.4	97.5	135.6	137.6	41.3
11	19.4	19.8	20.6	20.5	19.0	19.1	19.1	19.0	21.9	20.8	19.4	17.9	16.9	19.1
12	19.5	20.7	27.9	23.8	24.2	24.1	24.2	24.3	17.0	29.6	16.6	21.2	22.5	20.2
13	33.6	33.9	36.5	28.1	29.3	29.4	29.4	29.0	28.9	28.9	30.1	26.4	26.4	31.5
14	16.0	17.5	26.0	17.4	18.5	18.3	18.5	17.8	22.6	23.6	19.6	15.8	15.9	203.4
15	66.5	66.3	68.4	79.0	69.3	69.3	69.3	74.9	71.2	70.3	78.1	77.6	78.2	81.2
OCH_3	54.1	54.8				55.9								
CH_3CO		170.7 170.2								179.5				
CH_3CO		20.8 21.2								21.3				
OCH_2CH_3							64.3							
OCH_2CH_3							15.7							

[a] Spectra of compounds **1b**, **2**−**6**, and **8**−**11** were recorded in $CDCl_3$, compounds **1a** and **7** in CD_3OD. [b] 125 MHz. [c] 75 MHz. [d] 50 MHz. [e] From T. Kimata, et al.[6] [f] From I. G. Collado, et al.[8]

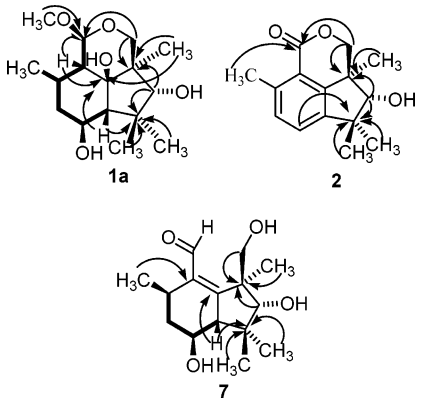

Figure 1. Selected ^{1}H−^{1}HCOSY (—) and HMBC (H→C) correlations of compounds **1a**, **2**, and **7**.

chemical shifts except for C-7. The above information suggested compound **2** to be 7-hydroxy-10-oxodehydrodihydrobotrydial.

The ^{1}H, ^{13}C, and DEPT NMR spectra of compound **3** were similar to those of **2** (Tables 1, 3), suggesting that **3** has a similar aromatic part. The only difference was the replacement of the carbonyl group at C-10 by a hydroxyl group. In the ^{13}C NMR spectrum, the signal for C-10 was shifted upfield (δ 92.3) compared to that of **2** (δ 164.0). The

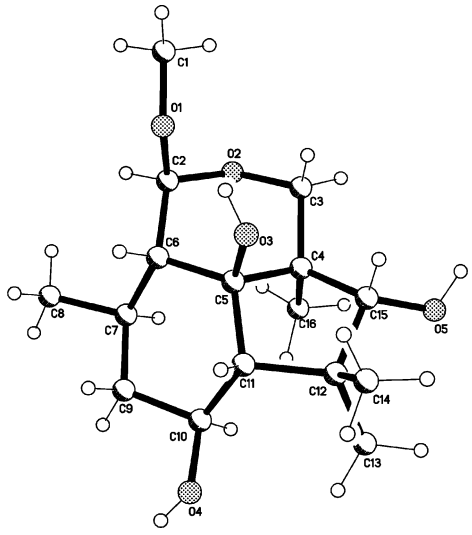

Figure 2. Structure of compound **1a** in the crystalline state.

structure of **3** was therefore elucidated as 7,10-dihydroxy-dehydrodihydrobotrydial.

Compound **4** was obtained as a colorless resin with the molecular formula $C_{16}H_{22}O_3$, as deduced from high-resolution mass spectral data. Comparison of the NMR spectra

of **4** (Tables 1, 3) with those of **3** revealed that **4** had a similar structure. The only difference was that the hydroxyl group at C-10 was replaced by a methoxyl group. The signal for C-10 in the ^{13}C NMR spectrum for **4** (δ 96.1) was shifted slightly but characteristically downfield compared to that of **3** (δ 92.3). The structure of **4** was therefore elucidated as 7-hydroxy-10-methoxydehydrodihydrobotrydial.

Compound **5** was also obtained as a colorless gum with the molecular formula $C_{17}H_{24}O_3$, as deduced from the mass spectral data. The ^1H, ^{13}C, and DEPT NMR spectra of compound **5** (Tables 1, 3) were related to those of **4**. However, the methoxy group at C-10 was replaced by an ethoxy group. The ^1H NMR signals and coupling patterns of **3**, **4**, and **5** in the top part of the molecule corresponded to those of **1a**, and the NOESY spectrum exhibited a correlation between 10-OCH_3 and H-15β, showing that these protons are on the same side of the molecule. Thus, the OR group at C-10 in **3**, **4**, and **5** also has the β-configuration. The structure of **5** was therefore determined as 7-hydroxy-10-ethoxydehydrodihydrobotrydial.

The molecular formula of compound **6**, $C_{15}H_{20}O_2$, was deduced from mass spectral (*m/z* 232) and ^{13}C NMR spectroscopic data. The IR and ^1H and ^{13}C NMR spectra (Tables 2, 3) of compound **6** indicated that it was related to compounds **3**−**5**. The major differences were the presence of two doublets for a benzylic methylene group at δ_H 4.61, 4.94 (J = 15.8 Hz) and the absence of signals corresponding to the proton and OR at C-10. Thus, compound **6** is named 7-hydroxy-10-dehydroxydehydrodihydrobotrydial.

The next two compounds existed as the interconvertible hydroxymethylaldehyde **7** and the cyclic hemiacetal **8**. However, by rapid preparative TLC, they could be separated and characterized by their NMR spectra. Compound **7** was obtained as colorless crystals with the molecular formula $C_{15}H_{24}O_4$, as deduced from mass spectral and ^{13}C NMR spectroscopic data. Its IR spectrum showed strong absorptions for hydroxyl groups (3331 cm^{-1}), while the ^1H NMR spectrum (Table 2) exhibited the presence of four methyl groups, of which three were singlets (δ 0.86, 1.27, and 1.29) and one was a doublet (δ 1.10, d, J = 6.7 Hz). The spectrum further showed four signals for protons on oxygenated carbons at δ 3.64 (m), 3.66 (s), 3.40 (d, J = 10.0 Hz), 3.58 (d, J = 10.0 Hz), and one low-field aldehyde signal (δ 10.27). The ^{13}C NMR spectrum of **7** (Table 3) showed signals for 15 carbons, and the DEPT spectra indicated the presence of four methyls, two methylenes, five methines, and four quaternary carbons. Comparison of the ^{13}C NMR spectroscopic data with those of botryenalol (**7a**),[4,5] a sesquiterpene with the botrydial skeleton, showed the identity of the chemical shifts except for the signal for C-7. This suggested that compound **7** was another botrydial sesquiterpene with a hydroxyl group located on C-7. Analysis of the COSY and HMQC spectrum of **7** enabled the deduction of the fragment −CH(2)(CH_3 (11))−CH_2(3)−CH(4)−CH(5)−. In the HMBC spectrum of **7**, ^{13}C−^1H long-range correlation signals were found between C-1 and H-2, H-10, H-11; C-6 and H-4, H-5, H-7, H-12, H-13; C-8 and H-5, H-7, H-14, H-15; and C-9 and H-5, H-10, H-14 (Figure 1). Therefore, the structure of **7** was determined as 7-hydroxydeacetylbotryenalol. The NMR spectral data of botryenalol (**7a**) are listed in Tables 2 and 3 for comparison.

Compound **8** was obtained as a colorless gum with the molecular formula $C_{15}H_{24}O_4$, as deduced from the mass spectral and ^{13}C NMR spectroscopic data. Analysis of the ^{13}C NMR spectrum (Table 3) by a DEPT experiment revealed the presence of 15 carbons: four methyls, two methylenes, five methines, and four quaternary carbons atoms. Parts of the ^1H NMR spectrum (Table 2) were similar to those of compound **7**. The major differences were the presence of a proton (δ 5.22 s) on an oxygenated carbon and the absence of the aldehyde signal. The remaining signals in the ^{13}C NMR spectrum along with the chemical shift and coupling constants appearing in the ^1H NMR spectrum were consistent with parts of the structure of compound **7**. In the HMBC spectrum of **8**, ^{13}C−^1H long-range selected correlations were found between C-6 and H-4, H-5, H-7, H-12, H-13; C-8 and H-5, H-7, H-14, H-15; C-9 and H-5, H-7, H-14, H-15; and C-10 and H-15. The ^1H NMR signals and coupling patterns of **8** in the top part of the molecule corresponded exactly to those of **3**. Thus, the OH group at C-10 also has the β-configuration. The above information suggested compound **8** to be 7,10-dihydroxydeacetyldihydrobotrydial-1(10)-ene. Upon standing in solution, the slow conversion of hemiacetal **8** to **7** and other decomposition products could be observed by TLC.

Analysis of the ^{13}C NMR spectrum by a DEPT experiment of compound **9** also revealed the presence of 15 carbons: four methyls, three methylenes, three methines, and five quaternary carbon atoms. The mass spectrum showed a [M^+] peak (*m/z* 234), consistent with the molecular formula $C_{15}H_{22}O_2$. Relevant parts of the ^1H NMR spectrum were similar to those of compound **1a**. The major differences were the presence of an olefinic proton (δ 6.15, s) and the absence of signals corresponding to the protons and methoxyl groups at C-1, C-4, C-5, and C-10, suggesting that **9** was a 1,3-diene. This was confirmed by the presence of signals at δ 114.6, 133.5, 135.5, and 137.6 in the ^{13}C NMR spectrum, which were assigned to C-1, C-5, C-9, and C-10, respectively. Analysis of the COSY and HMQC spectrum of **9** enabled the deduction of the fragment −CH(2)(CH_3(11))−CH_2(3)−CH_2(4)−. In the HMBC spectrum of **9**, ^{13}C−^1H long-range selected correlations were found between C-6 and H-4, H-7, H-12, H-13; C-8 and H-7, H-14, H-15; C-9 and H-2, H-7, H-14, H-15; and C-1 and H-10, H-2, H-11. Thus, compound **9** is named 4,10-didehydroxy-7-hydroxydeacetyldihydrobotrydial-1(10),5(9)-diene.

1D (^1H, ^{13}C, and DEPT) and 2D (COSY and HMQC) NMR spectra of compound **10** were quite similar to those of **9**, except for the appearance of a hydroxyl group at C-4. Analysis of the COSY and HMQC spectrum of **10** enabled the deduction of the fragment −CH(2)(CH_3(11))−CH_2(3)−CH(4)−. Therefore, the structure of **10** was determined as 7-hydroxy-10-dehydroxydeacetyldihydrobotrydial-1(10),5-(9)-diene.

Compound **11** was obtained as a colorless gum. The molecular formula, $C_{15}H_{22}O_2$, was deduced from the mass spectrum (*m/z* 234) and ^{13}C NMR spectroscopic data. The IR absorption at 3410 cm^{-1} and ^{13}C NMR signal at δ 81.2 indicated the presence of a hydroxyl group in the molecule. Its ^{13}C NMR spectrum (Table 3) exhibited signals for three methyls (δ 19.1, 20.2, and 31.5), three methylenes (δ 41.3, 50.3, and 54.0), and six methines (δ 30.7, 34.9, 42.0, 81.2, 136.1, and 203.4), as well as three quaternary carbons (δ 50.6, 58.1, and 151.3). Moreover, the ^1H NMR spectrum (Table 2) showed the presence of three methyl groups, of which two were singlets (δ 1.09 and 1.20) and one was a doublet (δ 1.00, d, J = 6.3 Hz), one proton on an oxygenated carbon at δ 3.75 (dd, J = 10.1, 7.4 Hz), one alkene proton at δ 5.15 (brs), and an aldehyde signal (δ 9.77 s). These data suggest that **11** is an unsaturated tricarbocyclic sesquiterpenoid of the probotryane type, displaying both an aldehyde and a secondary hydroxyl group. The COSY and HMQC spectra of **11** showed the presence of its coupled

proton systems as CH(15)−CH_2(10)−CH(1)(CH (9))−CH-(2)(CH_3(11))−CH_2(3). The ^{1}H and ^{13}C NMR spectrum of compound **11** was very similar to probotryane-4β,9β-diol, another sesquiterpene with the probotryane skeleton, isolated from *Botrytis cinerea*.[6] The main differences were the presence of an aldehyde group at C-8 and a double bond at C-4(5) and the absence of the hydroxy signal at C-9. The above information suggested the ring-contracted compound **11** to be 15-hydroxy-14-aldehyde probotryan-4(5)-ene.

The structures of further compounds, **12**, **13**, **14**, and **15**, isolated from *Geniculosporium* sp. 6580 were identified as L-696,474,[8] cytochalasin U,[8] RKS-1778,[9] and cytochalasin H[10] by comparing their NMR data with those reported in the literature.

Secondary metabolite production and activity may provide information both on the fungal−host interaction and on adaptations to the microenvironment. As shown in Tables 4 and 5,[12] these novel botryane metabolites have some inhibitory activity against the test organisms used in these studies: *Chlorella fusca*, *Bacillus megaterium*, and *Microbotryum violaceum*. The fungicidal and antibacterial activities could play a role in the association by defending the host from microbial pathogens. The algicidal activities could be important for maintaining a balance between the antagonisms of host and fungus, as has been hypothesized for plant endophytes with their hosts.[13]

Experimental Section

General Experimental Procedures. For general methods and instrumentation see ref 14, and for microbiological methods and conditions of culture see ref 15. Melting points were determined on a GALLENKAMP micro-melting point apparatus and are uncorrected. NMR spectra were run on Bruker Avance-500, -300, and AXR-200 NMR spectrometers with TMS as internal standard. EIMS were obtained on a MAT 8200 mass spectrometer.

Extraction and Isolation. The marine fungus *Geniculosporium* sp., belonging to the Xylariaceae, was isolated from the red alga *Polysiphonia* sp. from the Baltic Ocean, near Ahrenshoop, Germany, and was cultivated at room temperature for 21 days on biomalt solid agar media. The culture media were then extracted with ethyl acetate to afford 20.5 g of a residue after removal of solvent under reduced pressure. The extract was separated into three fractions by column chromatography (CC) on silica gel (400 g), using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The less polar fraction 1 (7.8 g) contained mainly fatty acids and lipids. The remaining two fractions were each further purified by silica gel column chromatography (CC), preparative TLC, and Sephadex (LH-20). The next fraction (4.2 g) was separated by CC over 200 g of silica gel with hexane/ethyl acetate (10:1, 1000 mL, 5:1, 1000 mL) to give two subfractions, A and B. Fraction A (800 mg) was separated by CC over 10 g of silica gel with hexane/ethyl acetate (7:1, 550 mL) to give crude **2**, **3**, **5**, and **6**. Fraction B (850 mg) was separated by CC over 10 g of silica gel with hexane/ethyl acetate (4:1, 450 mL) to give crude **9**, **10**, **11**, and **12**. Subsequently, each crude fraction was further purified by preparative TLC chromatography on silica gel (1 mm, Macherey and Nagel) and Sephadex (LH-20) to give compounds **2** (8 mg), **3** (7 mg), **5** (15 mg), **6** (17 mg), **9** (6 mg), **10** (5 mg), **11** (8 mg), and **12** (20 mg). The more polar fractions (5.1 g) were separated by silica gel column chromatography eluted with dichloromethane/ethyl acetate (6:1, 980 mL) to give crude crystals of **1a**, **7**, and **13**, successively. The samples were then recrystallized from MeOH to give **1a** (70 mg), **7** (10 mg), and **13** (10 mg). The remaining fraction was further subjected to silica gel column chromatography eluted with dichloromethane/ethyl acetate (7:1, 580 mL) to give pure compounds **4** (5 mg), **8** (11 mg), **14** (15 mg), and **15** (8 mg).

X-ray Crystal Structure Determination of 1a. $C_{16}H_{30}O_6$, H_2O, M_r = 318.4, colorless crystal, size 0.50 × 0.12 × 0.12 mm^3, T = 120(2) K, orthorhombic, space group $P2_12_12_1$, a = 6.3385(4) Å, b = 13.3235(9) Å, c = 19.477(1) Å, V = 1644.8(2) Å^3, Z = 4, D_c = 1.286 g/cm^3, μ = 0.097 mm^{-1}, F(000) = 696. Data collection was performed with a Bruker SMART APEX CCD[16] using graphite-monochromated Mo Kα radiation, 22 921 intensities collected 1.85° ≤ θ ≤ 28.35°, h ±8, k ±17, l −25/26. The compound crystallizes in a non-centrosymmetric space group; however, in the absence of significant anomalous scattering effects, the Flack[17] parameter is essentially meaningless. Accordingly, Friedel pairs were merged. 23 69 independent reflections, R_{int} = 0.055. Structure solution[15] was by direct and Fourier synthesis, full-matrix least-squares refinement[15] based on F^2 and 214 parameters, all but H atoms were refined anisotropically, one solvent water molecule per asymmetric unit. Hydrogen atoms were refined at calculated positions riding on the carbon atoms with isotropic displacement parameters U_{iso}(H) = 1.2U_{eq}(C) or 1.5U_{eq}(−CH_3/−OH). All CH_3 and OH groups were allowed to rotate but not to tip. The H atoms of the water molecule were located from a difference map and refined freely. Refinement converged at R1 ($I > 2\sigma(I)$) = 0.039, wR2 (all data) = 0.097, min./max. height in final ΔF = −0.17/0.31 e/Å^3. Figure 2 shows the molecular structure of **1a**. Crystal packing is determined by strong intermolecular hydrogen bonds O4−H4···O5 (−x, y−0.5, −z+0.5) with H4···O5 2.049(2) Å, O5−H5···O100 (x, y, z) with H5···O100 1.954(2) Å, O100−H102···O2 (x+0.5, −y+0.5, −z+1) with H102···O2 2.02(3) Å, and O100−H101···O4 (−x+1, y+0.5, −z+0.5) with H101···O4 1.87(3) Å.

Full crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-239851. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

7-Hydroxy-10-methoxydeacetyldihydrobotrydial (1a): colorless crystals (MeOH); mp 145−146 °C (MeOH); [α]$^{25}_D$ +55 (*c* 0.016, MeOH); IR (KBr) ν_{max} (film) 3500, 3420, 2965, 2365, 1563, 1382, 1062, 1010, 668, 544 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 268 [M − CH_3OH] (48), 250 (69), 222 (60), 192 (100), 163 (48), 149 (79), 109 (79), 83 (92), 43 (40), 28 (38).

7-Acetyl-10-methoxydeacetyldihydrobotrydial (1b). 1a (10 mg) was dissolved in pyridine (1 mL), and acetic anhydride (0.5 mL) was added. The reaction mixture was stirred for 15 h at ambient temperature. The solvent was evaporated at reduced pressure to afford crude **1b**, which was purified by preparative TLC using dichloromethane/ethyl acetate (5:1), yielding **1b** as a colorless gum: [α]$^{25}_D$ +13.2 (*c* 0.05, CH_2Cl_2); ^{1}H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 352 [M − CH_3OH] (25), 292 (60), 233 (60), 189 (62), 174 (90), 113 (56), 95 (100), 43 (60), 29 (15).

7-Hydroxy-10-oxodehydrodihydrobotrydial (2): colorless gum; [α]$^{25}_D$ +2.5 (*c* 0.02, CH_2Cl_2); IR (KBr) ν_{max} (film) 3431, 2975, 1700, 1237, 1072, 1020 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HREIMS *m/z* 246.1249 (calcd for $C_{15}H_{18}O_3$ 246.1256).

7,10-Dihydroxydehydrodihydrobotrydial (3): colorless gum; [α]$^{25}_D$ +37.0 (*c* 0.02, CH_2Cl_2); IR (KBr) ν_{max} (film) 3462, 2960, 2862, 1455, 1087, 1036, 813 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 230 [M$^+$ − H_2O] (100), 217 (75), 195 (49), 159 (60), 143 (40), 128 (38), 43 (75).

7-Hydroxy-10-methoxydehydrodihydrobotrydial (4): colorless gum; [α]$^{25}_D$ +64.3 (*c* 0.05, CH_2Cl_2); IR (KBr) ν_{max} (film) 3456, 2965, 1636, 1465, 1067, 813 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; HREIMS *m/z* 262.1569 (calcd for $C_{16}H_{22}O_3$ 262.1568).

7-Hydroxy-10-ethoxydehydrodihydrobotrydial (5): colorless gum; [α]$^{25}_D$ +67.8 (*c* 0.03, CH_2Cl_2); IR (KBr) ν_{max} (film) 3461, 2966, 2928, 2868, 1090, 1041 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; EIMS *m/z* 276 [M$^+$] (70), 246 (45), 231 (100), 217 (30), 205 (20), 88 (35), 70 (50).

7-Hydroxy-10-dehydroxydehydrodihydrobotrydial (6): colorless gum; [α]$^{25}_D$ −20.4 (*c* 0.03, CH_2Cl_2); IR (KBr) ν_{max} (film) 3445, 2955, 2923, 2868, 1699, 1074, 1025 cm^{-1}; ^{1}H and ^{13}C

NMR data, see Tables 2 and 3; EIMS *m/z* 232 [M^+] (45), 202 (50), 187 (65), 159 (20), 84 (100), 49 (80).

7-Hydroxydeacetylbotryenalol (7): colorless crystals (MeOH); mp 200−201 °C; $[\alpha]^{25}_D$ +78.3 (*c* 0.02, MeOH); IR (KBr) ν_{max} (film) 3331, 2928, 2852, 1726, 1650, 1014 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 268 [M^+] (10), 250 (30), 238 (70), 191 (100), 177 (45), 149 (60), 91 (20), 43 (65).

7,10-Dihydroxydeacetyldihydrobotrydial-1(10)-ene (8): colorless gum; $[\alpha]^{25}_D$ +67.9 (*c* 0.02, CH_2Cl_2); IR (KBr) ν_{max} (film) 3423, 2955, 2923, 2858, 1112, 1068, 1025 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 268 [M^+] (15), 251 (25), 208 (30), 189 (20), 84 (100), 49 (95).

4,10-Didehydroxy-7-hydroxydeacetyldihydrobotrydial-1(10),5(9)-diene (9): colorless gum; $[\alpha]^{25}_D$ +7.0 (*c* 0.01, CH_2Cl_2); IR (KBr) ν_{max} (film) 3420, 2924, 1724, 1465, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 234 [M^+] (5), 231 (100), 217 (20), 173 (15), 119 (10), 83 (13), 43 (38).

7-Hydroxy-10-dehydroxydeacetyldihydrobotrydial-1(10),5(9)-diene (10): colorless gum; $[\alpha]^{25}_D$ +29.0 (*c* 0.18, CH_2Cl_2); IR (KBr) ν_{max} (film) 3431, 2924, 2856, 1713, 1450, 1067 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 250 [M^+] (5), 232 (10), 192 (80), 159 (40), 149 (80), 91 (48), 43 (100), 29 (25).

15α-Hydroxy-14-aldehyde probotryan-4 (5)-ene (11): colorless gum; $[\alpha]^{25}_D$ +11.7 (*c* 0.06, CH_2Cl_2); IR (KBr) ν_{max} (film) 3410, 2960, 1724, 1641, 1460, 1056 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; EIMS *m/z* 234 [M^+] (15), 232 (14), 218 (21), 192 (89), 177 (48), 159 (49), 149 (47), 121 (48), 107 (79), 91 (52), 69 (70), 43 (100), 29 (38).

Acknowledgment. We thank BASF AG and the BMBF (Bundesministerium für Bildung und Forschung, project no. 03F0360A) for financial support.

Supporting Information Available: Tables of relative inhibition of algal growth by compounds **1**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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NP0498206