Chemical Transformations on Botryane Skeleton. Effect on the Cytotoxic Activity

José L. Reino,[†] Rosa Durán-Patrón,[†] Inmaculada Segura,[‡] Rosario Hernández-Galán,[†] Hans H. Riese,[‡] and Isidro G. Collado*[,†]

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain, and Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología (CSIC), UAM Campus Cantoblanco, Madrid, Spain

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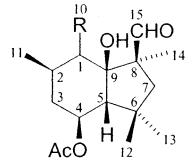
Eighteen compounds with a botryane skeleton have been obtained through chemical transformations of various toxins from the fungus *Botrytis cinerea*. During the course of these transformations, the C-10 carbon of the botryane skeleton was found to exhibit an interesting high regioselectivity to oxidizing and reducing agents. In addition, the cytotoxicity of 27 botryane derivatives was determined in vitro against Hs578T, MDA-MB-231, HT-1080, U87-MG, IMR-90, and HUVEC cell lines. The results of this study confirm that the cytotoxicity of botrydial (**1**) and its derivatives is related to the presence of a 1,5-dialdehyde functionality.

The botryane skeleton is a bicyclic sesquiterpenoid, non-isoprenoid system, which has been the subject of many investigations, especially concerning its biosynthetic pathway. In a series of noteworthy papers, Hanson and Bradshaw[1−3] described the way in which farnesyl pyrophosphate is folded to generate the botryane skeleton; however, the chemical behavior of this interesting carbon skeleton has not yet been reported. The most representative compounds with a botryane skeleton to date are botrydial (**1**)[4] and its derivatives, which are characteristic metabolites of the phytopathogenic fungus *Botrytis cinerea* Pers. (Sclerotiniaceae). These compounds have been found to display a wide range of biological activities. For instance, they are responsible for the typical lesions associated with *B. cinerea* infection, and they play an important role in the pathogenicity of the organism in vivo.[5−9] Botrydial (**1**) has been found to be particularly toxic, showing phytotoxic activity in vitro[6] and in planta[9] on both tobacco and bean leaves at a concentration of only 1 ppm. Compound **1** also exhibits an interesting antibiotic activity against *Bacillus subtillus* and *Phytium debaryanum* at 100 ppm, as well as a high cytotoxic activity against tumoral and nontumoral cells.[4,7] These toxins have thus far been isolated only in quantities that were too small to permit chemical transformations; therefore, the reactivity of this skeleton remains practically unexplored.

In this paper we describe various chemical transformations carried out on botrydial (**1**) and dihydrobotrydial (**2**). These experiments not only have yielded 18 derivatives (**5** and **12**−**28**) but also have allowed us to draw interesting conclusions concerning botryane skeleton reactivity. The cytotoxicity of 17 of the compounds (**5**, **12**−**16**, and **18**−**28**), along with that of 11 compounds isolated from *B. cinerea* (**1**−**11**), has also been studied. The results of the bioassays shed light on the relationship between the structural changes and the cytotoxic activity of the toxins.



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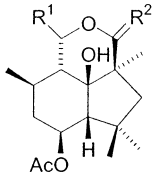
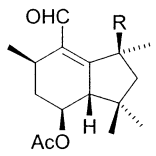
3: R = β-CHO



2: R^1 = β-OH; R^2 = H, H
4: R^1 = α-OH; R^2 = H, H
11: R^1 = H; R^2 = O



5: R = CHO
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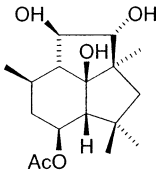

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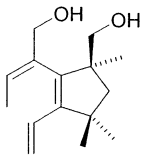

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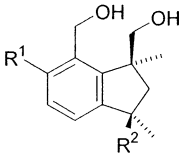

9: R^1 = CH_2OH; R^2 = CH_3
10: R^1 = CH_3; R^2 = CH_2OH

Results and Discussion

Botrydial (**1**)[4] and dihydrobotrydial (**2**),[4] which served as the starting material for the chemical transformations, were isolated from the strain *B. cinerea* 2100, obtained from Colección Española de Cultivos Tipo (CECT). The fungus was cultured on Czapek-Dox medium, which was shaken for 3 days. The fermentation broth was extracted with ethyl acetate as described in the Experimental Section. Chromatography of the extract on silica gel, followed by final purification by means of HPLC (normal phase, petroleum/

* To whom correspondence should be addressed. Tel: +34-956-016368. Fax: +34-956-016193. E-mail: isidro.gonzalez@uca.es.
[†] Universidad de Cádiz.
[‡] Centro Nacional de Biotecnología.

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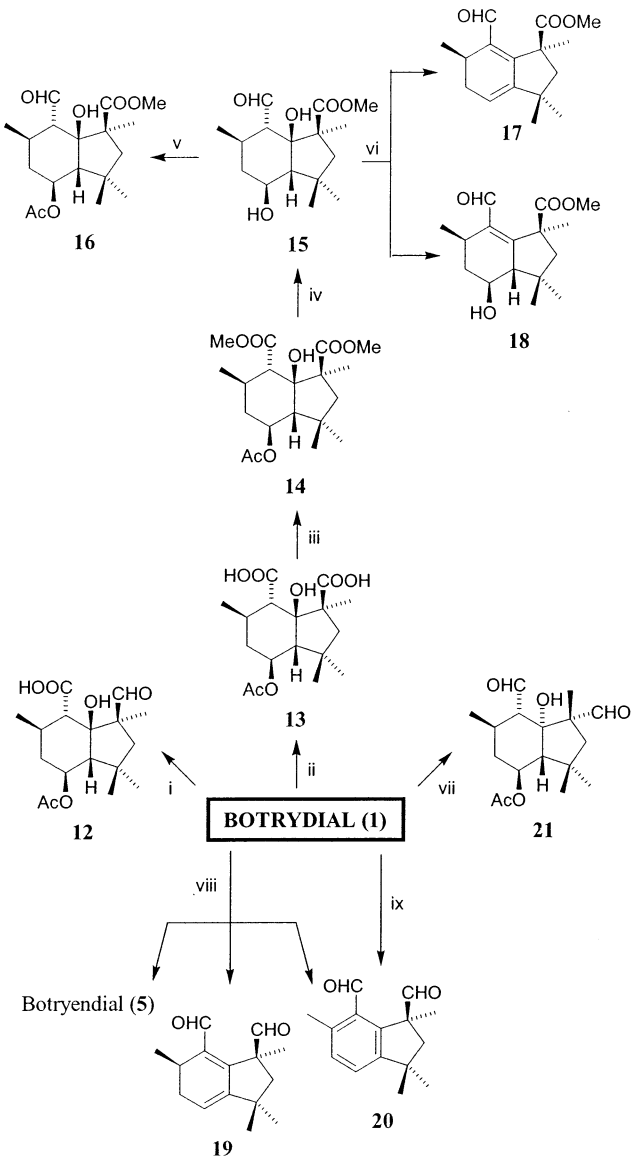
Scheme 1. Chemical Transformations on Botrydial (**1**)[a]



[a] (i) 8 N CrO_3, rt; (ii) $KMnO_4$, rt; (iii) CH_2N_2, rt; (iv) DIBAL, rt; (v) Ac_2O, Py, rt; (vi) 6% oxalic acid, reflux; (vii) toluene, reflux; (viii) 6% oxalic acid, rt; (ix) *p*-TsOH, reflux.

ethyl acetate), led to the isolation of **1** and **2**, as well as the known compounds **3**−**10**,[5,7,8,10] in sufficient amounts for the cytotoxic assays. Lactone **11** was obtained from a static culture of *B. cinerea* that had previously been carried out by our research group.[5]

To obtain derivatives oxidized at C-10 and/or C-15, different oxidizing reagents were used to treat botrydial (**1**), which showed an interesting high regioselectivity on C-10. Treatment of **1** with 8 N CrO_3 (see Scheme 1) afforded a crystalline compound that showed an $[M - H_2O]^+$ peak at *m*/*z* 308 and a ^{13}C NMR spectrum consistent with the molecular formula $C_{17}H_{26}O_6$. The absence of the characteristic doublet for H-10 in the ^1H NMR spectrum and the appearance of a signal at δ 176.6 in the ^{13}C NMR spectrum were both consistent with an oxidization at C-10 yielding **12**. However, as we have previously reported,[7] when botrydial (**1**) was treated with $KMnO_4$, carbon atoms 10 and 15 were both oxidized, yielding **13**. These results indicate a greater reactivity for the aldehyde group on C-1 in relation to that on C-8 in the botrydial (**1**) skeleton. This feature was used to prepare botrydial derivatives modified on only one aldehyde group. In this way, botrydioic acid (**13**) was methylated with diazomethane to afford the dimethyl ester **14**,[7] which was then regioselectively reduced with DIBAL to the monoaldehyde **15** and subsequently acetylated with acetic anhydride in basic medium to yield **16** (see Scheme 1). The structures of **15** and **16** were determined by means of NMR spectroscopy. All the data were in accordance with that published for methyl botryaloate (**15**) and methyl acetylbotryaloate (**16**), which were isolated from *B. cinerea* after methylation of the acid fraction.[11]

In another transformation, an organic solution of 1**5** was refluxed with 6% oxalic acid, yielding two unsaturated derivatives, **17** and **18** (see Scheme 1). The spectroscopic data for compound **17** was in agreement with that previously described for methyl botrydienaloate.[5] The structure of **18** was also inferred from its spectroscopic data. An $[M]^+$ peak at *m*/*z* 280 in the mass spectrum, as well as two singlets at δ 137.0 (C-1) and 164.1 (C-9) in the ^{13}C NMR spectrum, plus the deshielding of the signals corresponding to H-11, H-5, and H-2 were all consistent with methyl botryenaloate.

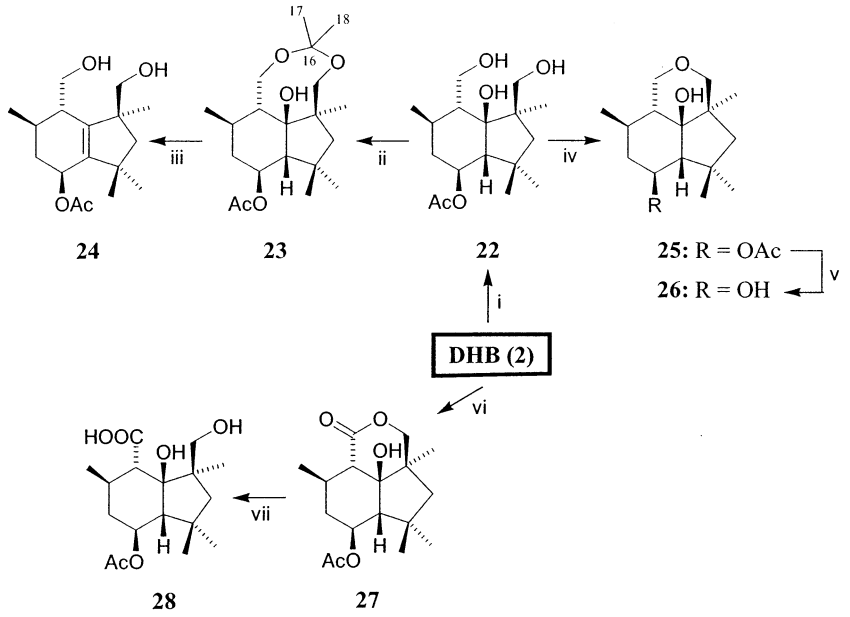
To obtain dehydro derivatives containing the 1,5-dialdehyde functionality, botrydial (**1**) was stirred with 6% oxalic acid for 3 days at room temperature to afford compounds **5**,[5] **19**,[12] and **20**.[12] However, when **1** was refluxed with *p*-toluenesulfonic acid, the aromatic dialdehyde **20**[12] was obtained quantitatively.

An unexpected epimerization of **1** at C-8 and C-9 occurred under reflux with dry toluene as solvent (see Scheme 1). Compound **21** was identified as the 8,9-epimer of **1**, previously isolated from *B. cinerea*,[7] using spectroscopy. The mechanism of this epimerization may be accounted for by a retro-aldol reaction followed by aldol reaction. Attack of the *re*-face of the enolate carbon nucleophile at the *si*-face of the carbonyl would lead to inversion of the configuration at C-9. A study using a semiempirical calculation[13] revealed that the heat of formation of the epimer with the aldehyde group at C-8 in the α position (−175.6 kcal/mol) was lower than that of the opposite epimer (−173.6 kcal/mol).

Reduction of dihydrobotrydial (**2**)[4] gave the acetoxytriol **22** (see Scheme 2). The primary hydroxyl groups of this compound were protected with acetone in acid medium to afford the parent acetonide **23**. The ^1H NMR spectrum of compound **23** showed two signals at δ 1.34 and 1.41 (3H each, s), which were correlated with the signals at δ 25.0 (q) and 24.5 (q) by means of HETCOR experiments. These spectral data, along with a new singlet at δ 101.1 in the ^{13}C NMR spectrum, confirmed the presence of a dimethylmethylendioxy moiety in **23**. In all probability, this moiety connects the hydroxyl groups at the C-10 and C-15 of **22**, as inferred from the upfield shift of the H-10, H-10′, and H-15 signals. Treatment of **23** with thionyl chloride/pyridine and subsequent workup with copper sulfate gave the unsaturated derivative botry-5(9)-enediol (**24**) (see Scheme 2). The structure of **24** was deduced by comparing its ^1H NMR spectrum with that of **22**. The spectra were similar, but the deshielded signals at δ 2.17 (H-1) and 5.42 (H-4) in **24**, as well as the observed change in the multiplicity of the H-4 signal (ddd in **22** to dd in **24**), indicated the presence of a double bond between C-9 and C-5 in **24**. This assignment was corroborated by the appearance of two signals at δ 143.6 (s) and 125.0 (s) in the ^{13}C NMR spectrum.

Compound **25**[4] was synthesized through dehydration of **22** with 6% oxalic acid. Subsequent hydrolysis of **25** with potassium carbonate gave the deacetyl derivative **26**.[4]

Scheme 2. Chemical Transformations on Dihydrobotrydial (**2**)[a]



[a] (i) $NaBH_4$, rt; (ii) acetone, *p*-TsOH, rt; (iii) $SOCl_2$/pyridine, rt; (iv) 6% oxalic acid, rt; (v) 1 M K_2CO_3, reflux; (vi) CrO_3, Py, rt; (vii) 1 N K_2CO_3, rt.

Table 1. Cytotoxic Activity of Botrydial (**1**) and Its Derivatives[a]

	ID_{50} (μg/mL)[b]					
cellular lines	**1**	**3**	**5**	**19**	**20**	**21**
IMR-90[c]	10	10	>10	1	10	10
HUVEC[c]	1	10	10	1	5	5
HT-1080[d]	1	10	10	1	1	5
Hs578T[d]	1	5	10	1	1	1
U87-MG[d]	1	5	10	1	5	1
MDA-MB-231[d]	1	1	10	0.1	10	0.1

[a] ID_{50} > 10 μg/mL for compounds **2**, **4**, **6**−**16**, **18**, and **22**−**28**. [b] Standard deviation was always lower than 3%. [c] Nontumoral human cell lines. [d] Tumoral human cell lines.

In yet another series of transformations, dihydrobotrydial (**2**) was oxidized with chromium trioxide in pyridine[11] to afford the δ-lactone (**27**),[10] which was hydrolyzed with potassium carbonate to yield the parent hydroxy acid (**28**). Examination of the NMR spectrum of this acid showed that only the δ-lactone functionality, and not the acetate moiety, had been hydrolyzed under these conditions.

In view of the biological interest in this class of compounds, we undertook a study of the cytotoxic activities of botrydial (**1**) and its natural (**2**−**11**) and synthetic (**12**−**16** and **18**−**28**) derivatives. Although the cytotoxicity of botrydial (**1**) and dihydrobotrydial (**2**) has already been reported,[7] we reassayed these compounds in this investigation to obtain comparable results.

The cytotoxic activity of the above-mentioned compounds was evaluated in vitro with the MTT assay. The cells used included human breast ductal carcinoma (Hs578T) and adenocarcinoma (MDA-MB-231), fibrosarcoma (HT-1080), glioblastoma (U87-MG), embryonic fibroblasts (IMR-90), and human umbilical vein endothelial cells (HUVEC). The results of the assays (see Table 1) show that botrydial (**1**) and its epimers (**3** and **21**), as well as the unsaturated derivatives **5**, **19**, and **20**, were more active than the remaining derivatives (**2**, **4**, **6**−**16**, **18**, and **22**−**28**) in inhibiting cell line proliferation. A study of the structure−activity relationships for this class of compounds indicates that the structural characteristic that most increases the cytotoxic effect of these compounds is the presence of a 1,5-dialdehyde functionality. This is especially evident when the cytotoxic activities of botrydial (**1**) are compared to those of the derivatives with modified aldehyde groups at C-1 and/or C-8 (**2**, **4**, **6**−**16**, **18**, and **22**−**28**).

Interestingly, botrydial (**1**) and 8,9-epibotrydial (**21**) were more active than the epimer **3**, indicating that an *S* configuration of C-1 of the botryane skeleton seems to play an important role in the cytotoxic activity. These results are in agreement with and confirm the conclusions obtained from previous assays to determine the phytotoxic and antibiotic activities of this class of compounds.[7]

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Mattson Genesis spectrophotometer, series FTIR. Mass spectra were recorded on a GC−MS Thermoquest spectrometer, model Voyager. Additional general experimental procedures have been reported.[7]

Organism and Culture Conditions. The culture of *B. cinerea* 2100 was obtained from the Colección Española de Cultivos Tipo (CECT), Facultad de Biología, Universidad de Valencia, Spain. The fungus was grown at 24−26 °C in 40 Erlenmeyer flasks (500 mL), each containing 160 mL of Czapek-Dox medium. The pH of the medium was adjusted to 7.0, and each flask was inoculated with 40 mL of a suspension of a 48 h old culture. Cultures were incubated for 3 days on an orbital shaker at 250 rpm.

Extraction and Isolation. The broth (8 L) was acidified to pH 2.0 with HCl, saturated with NaCl, and extracted with EtOAc. The EtOAc extract was washed with $NaHCO_3$ and H_2O and dried over anhydrous Na_2SO_4. Evaporation of the solvent at reduced pressure gave a yellow oil that was separated by means of column chromatography, with an increasing gradient of EtOAc in petroleum ether. Final purification was carried out with HPLC to afford botrydial[4] (**1**, 626 mg), dihydrobotrydial[4] (**2**, 759 mg), 1-epibotrydial[7] (**3**, 41 mg), 10-epidihydrobotrydial[5] (**4**, 43 mg), botryenedial[5] (**5**, 129 mg), botryenalol[5] (**6**, 95 mg), 4β-acetoxy-9β,10β,15α-trihydroxyprobotrydial[10] (**7**, 231 mg), secobotrytrienediol[8] (**8**, 9 mg), 11-hydroxydehydrobotrydienol[8] (**9**, 3 mg), and 12-hydroxydehydrobotrydienol[8] (**10**, 1.4 mg).

15-Oxobotry-10-oic Acid (12). A solution of **1** (31 mg, 0.1 mmol) in acetone was treated with drops of an 8 N aqueous solution of CrO_3 and stirred vigorously for 5 h at room temperature. Excess reagent was destroyed with Na_2SO_3 (2

mg). The mixture was diluted with water, and the acetone was removed by blowing nitrogen over the solution. The resulting solution was acidified to pH 2 and extracted with EtOAc (40 mL, ×4). The solvent was evaporated and the crude extract chromatographed to yield **12** (21 mg, 0.064 mmol, 64%) as a white solid: mp 137−139 °C; $[\alpha]_D^{25}$ +13° (*c* 0.8, $CHCl_3$); IR (dry film) ν_{max} 3507, 2961, 2871, 1737, 1721, 1462, 1366, 1244, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.97 (3H, d, J_{11-2} = 6.1 Hz, H-11), 1.08 (1H, br d, $J_{3\beta-3\alpha}$ = 12.6 Hz, H-3β), 1.09 (3H, s, H-13), 1.32 (3H, s, H-12), 1.33 (1H, d, $J_{7\alpha-7\beta}$ = 12.6 Hz, H-7α), 1.42 (3H, s, H-14), 2.00 (1H, d, J_{5-4} = 11.3 Hz, H-5), 2.03 (3H, s, C*H*$_3$COO−), 2.04 (1H, m, H-2), 2.16 (1H, ddd, $J_{3\alpha-3\beta}$ = 12.6 Hz, $J_{3\alpha-2}$ = 3.3 Hz, $J_{3\alpha-4}$ = 4.5 Hz, H-3α), 2.34 (1H, d, $J_{7\beta-7\alpha}$ = 12.6 Hz, H-7β), 2.46 (1H, d, J_{1-2} = 12.5 Hz, H-1), 5.08 (1H, ddd, J_{4-5} = $J_{4-3\beta}$ = 11.3 Hz, $J_{4-3\alpha}$ = 4.5 Hz, H-4), 9.57 (1H, s, H-15); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 19.3 (q, C-14), 20.7 (q, C-11), 21.5 (q, *C*H$_3$COO−), 27.2 (q, C-13), 29.2 (d, C-2), 35.6 (q, C-12), 38.6 (t, C-3), 39.4 (s, C-6), 51.8 (t, C-7), 59.1 (s, C-8), 60.5 (d, C-1), 62.6 (d, C-5), 72.5 (d, C-4), 89.1 (s, C-9), 170.3 (s, CH$_3$*C*O−), 176.6 (s, C-10), 207.4 (d, C-15); EIMS *m*/*z* 308 [M − H_2O]$^+$ (0.7), 293 [M − H_2O − CH_3]$^+$ (1.7), 248 [M − AcOH − H_2O]$^+$ (1.0), 222 [M − AcOH − CO_2]$^+$ (1.5), 207 [M − AcOH − CO_2 − CH_3]$^+$ (1.2), 182 (15), 140 (100).

Botrydioic Acid (13). A solution of **1** (10 mg, 0.034 mmol) in EtOAc (3 mL) was treated with a solution of $KMnO_4$ (7 mg) in H_2O (150 μL) and stirred vigorously for 12 h at room temperature. Excess reagent was destroyed with Na_2SO_3 (2 mg). The mixture was diluted with H_2O, acidified to pH 2 with HCl, and extracted with EtOAc (30 mL, ×4). The solvent was evaporated to give **13** (12.6 mg, quantitative) as an amorphous solid: $[\alpha]_D^{25}$ +9° (*c* 1.3, $CHCl_3$); IR (dry film) ν_{max} 3445, 2964, 2919, 2873, 1732, 1457, 1386, 1246, 1089, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (3H, d, J_{11-2} = 6.2 Hz, H-11), 1.06 (1H, m, $J_{3\beta-3\alpha}$ = 12.5 Hz, $J_{3\beta-4}$ = 11.3 Hz, H-3β), 1.09 (3H, s, H-13), 1.29 (3H, s, H-12), 1.49 (3H, s, H-14), 1.58 (1H, d, $J_{7\alpha-7\beta}$ = 12.3 Hz, H-7α), 2.01 (1H, m, J_{2-1} = 12.6 Hz, J_{2-11} = 6.2 Hz, $J_{2-3\alpha}$ = 3.0 Hz, H-2), 2.02 (3H, s, C*H*$_3$COO−), 2.04 (1H, d, J_{5-4} = 11.3 Hz, H-5), 2.15 (1H, ddd, $J_{3\alpha-2}$ = 3.0 Hz, $J_{3\alpha-3\beta}$ = 12.5 Hz, $J_{3\alpha-4}$ = 5.0 Hz, H-3α), 2.37 (1H, d, $J_{7\beta-7\alpha}$ = 12.3 Hz, H-7β), 2.57 (1H, d, J_{1-2} = 12.6 Hz, H-1), 5.08 (1H, dt, $J_{4-3\alpha}$ = 5.0 Hz, $J_{4-3\beta}$ = J_{4-5} = 11.3 Hz, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.2 (q, C-11), 21.0 (q, C-14), 21.4 (q, *C*H$_3$COO−), 27.3 (q, C-13), 29.5 (d, C-2), 35.7 (q, C-12), 38 (t, C-3), 39.1 (s, C-6), 54.7 (s, C-8), 55.2 (t, C-7), 60.9 (d, C-5), 72.9 (d, C-4), 61.2 (d, C-1), 87.7 (s, C-9), 170.5 (s, CH$_3$*C*OO−), 180.7 (s, C*-10), 185.6 (s, C*-15) (*interchangeable); EIMS *m*/*z* 282 [M − AcOH]$^+$ (6), 264 [M − AcOH − H_2O]$^+$ (18), 249 [M − AcOH − H_2O − CH_3]$^+$ (9), 236 [M − AcOH − HCOOH]$^+$ (38), 220 [M − AcOH − H_2O − CO_2]$^+$ (49), 119 (100).

Dimethyl Botrydioate (14). A solution of diazomethane in Et_2O was added in excess to **13** (10 mg, 0.027 mmol). The resulting solution was stirred at room temperature for 10 min, after which time the solvent and excess diazomethane were evaporated to afford **14**[7] (11 mg, quantitative).

Methyl Botryaloate (15). A solution of **14** (50 mg, 0.135 mmol) in dry THF (15 mL) was cooled to −78 °C and treated with DIBAL (0.4 mmol). The mixture was stirred at room temperature for 12 h, diluted with brine, and filtered through a layer of Celite (1.5 cm). The solution was extracted with Et_2O (×3), and the solvent was evaporated. The reaction crude thus obtained was purified by means of normal-phase HPLC to give **15**[11] (25 mg, 0.084 mmol, 62%) as a white solid: mp 115−117 °C; $[\alpha]_D^{28}$ +67° (*c* 0.43, $CHCl_3$); IR (dry film) ν_{max} 3424, 2957, 2872, 2734, 1714, 1276, 1133 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, d, J_{11-2} = 6.2 Hz, H-11), 1.14 (1H, ddd, $J_{3\beta-3\alpha}$ = 13.2 Hz, $J_{3\beta-4}$ = 11.2 Hz, $J_{3\beta-2}$ = 13.2 Hz, H-3β), 1.30 (9H, s, H-12, H-13, H-14), 1.55 (1H, d, $J_{7\alpha-7\beta}$ = 12.7 Hz, H-7α), 1.74 (1H, d, J_{5-4} = 10.7 Hz, H-5), 1.98 (1H, m, H-2), 2.00 (1H, m, H-3α), 2.32 (1H, d, $J_{7\beta-7\alpha}$ = 12.7 Hz, H-7β), 2.66 (1H, dd, J_{1-10} = 2.9 Hz, J_{1-2} = 11.9 Hz, H-1), 3.68 (3H, s, C*H*$_3$OCO−), 3.92 (1H, ddd, J_{4-5} = 10.7 Hz, $J_{4-3\alpha}$ = 4.6 Hz, $J_{4-3\beta}$ = 11.2 Hz, H-4), 5.74 (1H, s, −O*H*), 9.85 (1H, d, J_{10-1} = 2.9 Hz, H-10).

Compounds 17 and 18. A solution of **15** (25 mg, 0.084 mmol) in THF (10 mL) was treated with 6% aqueous oxalic acid solution (6 mL) and refluxed for 3.5 h. The mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with Et_2O (×3). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed, and the mixture obtained was purified by means of normal-phase HPLC to afford **17**[5] (12 mg, 0.046 mmol, 54%) and **18** (8 mg, 0.029 mmol, 34%).

Methyl botryenaloate (18): oil; $[\alpha]_D^{26}$ +104° (*c* 2.4, $CHCl_3$); IR (dry film) ν_{max} 3448, 2952, 2872, 2757, 1730, 1675, 1452, 1243, 1088 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.00 (3H, s, H-13), 1.03 (3H, d, J_{11-2} = 6.8 Hz, H-11), 1.25 (3H, s, H-12), 1.32 (1H, ddd, $J_{3\beta-3\alpha}$ = 12.7 Hz, $J_{3\beta-2}$ = 10.3 Hz, $J_{3\beta-4}$ = 11.4 Hz, H-3β), 1.56 (3H, s, H-14), 1.65 (1H, d, $J_{7\alpha-7\beta}$ = 13.0 Hz, H-7α), 2.07 (1H, ddd, $J_{3\alpha-3\beta}$ = 12.7 Hz, $J_{3\alpha-2}$ = 6.8 Hz, $J_{3\alpha-4}$ = 4.0 Hz, H-3α), 2.19 (1H, d, $J_{7\beta-7\alpha}$ = 13.0 Hz, H-7β), 2.48 (1H, dd, J_{5-4} = 9.2 Hz, J_{5-2} = 3.6 Hz, H-5), 2.80 (1H, m, H-2), 3.68 (3H, s, C*H*$_3$OCO−), 3.70 (1H, ddd, $J_{4-3\alpha}$ = 4.0 Hz, $J_{4-3\beta}$ = 11.4 Hz, J_{4-5} = 9.2 Hz, H-4), 9.80 (1H, s, H-10); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.4 (q, C-11), 23.6 (q, C-13), 29.5 (q, C-14), 30.0 (d, C-2), 30.3 (q, C-12), 39.9 (s, C-6), 41.7 (t, C-3), 51.1 (s, C-8), 52.7 (q, *C*H$_3$OCO−), 56.0 (t, C-7), 61.6 (d, C-5), 68.5 (d, C-4), 137.0 (s, C-1), 164.1 (s, C-9), 176.7 (s, C-15), 191.9 (d, C-10); EIMS *m*/*z* 280 [M]$^+$ (0.3), 262 [M − H_2O]$^+$ (0.3), 252 [M − CO]$^+$ (8), 234 [M − H_2O − CO]$^+$ (9), 220 [M − HCOOCH$_3$]$^+$ (11), 205 [M − HCOOCH$_3$ − CH_3]$^+$ (9), 175 (100).

Compounds 5, 19, and 20. A solution of **1** (33 mg, 0.106 mmol) in EtOAc (5 mL) was treated with 6% aqueous oxalic acid solution (40 mL). After stirring for 72 h at room temperature, the mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc (40 mL, ×4). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed, and the mixture obtained was purified by means of normal-phase HPLC using hexane/EtOAc (17:3) to give **5**[5] (1.7 mg, 0.006 mmol, 5%), **19**[12] (14 mg, 0.060 mmol, 56%), and **20**[12] (10 mg, 0.043 mmol, 40%).

In addition, botrydial (**1**, 40 mg, 0.130 mmol) dissolved in EtOAc was treated with a catalytic amount of *p*-toluenesulfonic acid, and the mixture was stirred and refluxed for 3 h. The organic solution, after a workup similar to that described above for the oxalic acid reaction, afforded only **20**[12] (30 mg, 0.130 mmol, quantitative).

8,9-Epibotrydial (21). A solution of **1** (41 mg, 0.132 mmol) in dry toluene (13 mL) was refluxed and stirred for 12 h. The solvent was removed under reduced pressure to afford **21**[7] (41 mg, 0.132 mmol, quantitative).

Botrydiol (22). $NaBH_4$ (2 mg) was added to a solution of **2** (73 mg, 0.234 mmol) in methanol (4 mL), and the resulting solution was stirred for 2 h at room temperature. The mixture was poured onto ice, acidified with 2 N HCl (10 mL), and stirred for 10 min. The solution was diluted with H_2O (45 mL) and extracted with $CHCl_3$ (30 mL, ×3). The solvent was evaporated and the crude extract chromatographed to yield **22**[4] (39 mg, 0.124 mmol, 53%) as a white solid: mp 175−176 °C; $[\alpha]_D^{20}$ +7° (*c* 1.15, $CHCl_3$); IR (dry film) ν_{max} 3393, 3430, 1730, 1240 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (3H, d, J_{11-2} = 5.8 Hz, H-11), 1.01 (3H, s, H*-13), 1.05 (3H, s, H*-14), 1.07 (1H, m, $J_{3\beta-3\alpha}$ = 12.0 Hz, H-3β), 1.17 (1H, d, $J_{7\alpha-7\beta}$ = 13.2 Hz, H-7α), 1.22 (3H, s, H-12), 1.65 (1H, m, J_{1-10} = 3.3 Hz, H-1), 1.66 (1H, m, H-2 superimposed on H-1), 1.83 (1H, d, J_{5-4} = 11.2 Hz, H-5), 1.99 (3H, s, C*H*$_3$COO−), 2.08 (1H, ddd, $J_{3\alpha-2}$ = 2.5 Hz, $J_{3\alpha-3\beta}$ = 12.0 Hz, $J_{3\alpha-4}$ = 5.0 Hz, H-3α), 2.17 (1H, d, $J_{7\beta-7\alpha}$ = 13.2 Hz, H-7β), 3.59 (1H, d, $J_{15-15'}$ = 11.5 Hz, H-15), 3.68 (1H, d, $J_{15'-15}$ = 11.5 Hz, H-15′), 3.89 (1H, dd, $J_{10'-10}$ = 11.0 Hz, H-10′), 3.98 (1H, dd, J_{10-1} = 3.3 Hz, $J_{10-10'}$ = 11.0 Hz, H-10 superimposed on O*H*-), 3.97, 4.06, 4.82 (each 1H, br s, −O*H*), 5.04 (1H, ddd, $J_{4-3\alpha}$ = 5.0 Hz, $J_{4-3\beta}$ = 11.2 Hz, J_{4-5} = 11.2 Hz, H-4) (*interchangeable); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.5 (q, C-11), 21.5 (q, *C*H$_3$COO−), 22.6 (q, C*-14), 28.0 (q, C*-13), 28.5 (d, C-2), 35.9 (q, C-12), 36.6 (s, C-6), 40.6 (t, C-3), 50.4 (s, C-8), 53.8 (t, C-7), 55.9 (d, C-1), 60.5 (t, C-10), 64.7 (d, C-5), 68.0 (t, C-15), 73.4 (d, C-4), 90.1 (s, C-9), 170.6 (s, CH$_3$*C*OO−) (*interchangeable); EIMS *m*/*z* 296 [M − H_2O]$^+$ (82), 236 [M − AcOH]$^+$ (80), 200 (80), 140 (88), 96 (100).

Compound 23. A solution of **22** (30 mg, 0.096 mmol) in dry acetone (10 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid for 12 h at room temperature. The

acetone was removed by blowing nitrogen over the solution, and the product was recovered in EtOAc. The EtOAc extract was washed (×2) with aqueous $NaHCO_3$ and brine. The solvent was evaporated under reduced pressure, and the crude product was subjected to column chromatography to afford **23** (10.1 mg, 0.029 mmol, 30%) as a white solid: mp 93−95 °C; $[\alpha]_D^{25}$ +8° (*c* 0.65, $CHCl_3$); IR (dry film) ν_{max} 3506, 1737, 1243, 1211, 1066, 1017 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, s, J_{11-2} = 6.4 Hz, H-11), 1.03 (1H, d, $J_{7\alpha-7\beta}$ = 12.8 Hz, H-7α), 1.17 (3H, s, H-12), 1.20 (3H, s, H-13), 1.23 (3H, s, H-14), 1.34 (3H, s, H*−17), 1.40 (1H, q, $J_{3\beta-3\alpha}$ = 12.1 Hz, H-3*β*), 1.41 (3H, s, H*−18), 1.48 (1H, m, H-2), 1.72 (1H, d, $J_{7\beta-7\alpha}$ = 12.8 Hz, H-7*β*), 1.83 (1H, q, J_{1-2} = 6.6 Hz, H-1), 1.99 (1H, d, H-5), 2.00 (3H, s, C*H*$_3$COO− superimposed on H-5), 2.02 (1H, m, H-3α superimposed on H-5 and C*H*$_3$COO-), 2.96 (1H, d, $J_{15'-15}$ = 13.6 Hz, H-15′), 3.74 (1H, dd, $J_{10'-10}$ = 10.6 Hz, $J_{10'-1}$ = 6.6 Hz, H-10′), 3.82 (1H, dd, $J_{10-10'}$ = 10.6 Hz, J_{10-1} = 6.3 Hz, H-10), 3.83 (1H, d, $J_{15-15'}$ = 13.6 Hz, H-15), 4.22 (1H, s, −O*H*), 5.02 (1H, dt, $J_{4-3\alpha}$ = 4.8 Hz, $J_{4-3\beta}$ = J_{4-5} = 11.4 Hz, H-4), (*interchangeable); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.4 (q, C-11), 21.5 (q, *C*H$_3$COO-), 21.9 (q, C-14), 24.5 (q, C*−18), 25.0 (q, C*−17), 27.8 (q, C-13), 30.0 (d, C-2), 36.0 (q, C-12), 36.3 (s, C-6), 40.0 (t, C-3), 51.7 (s, C-8), 52.2 (d, C-1), 55.1 (t, C-7), 62.4 (t, C-10), 63.1 (d, C-5), 66.6 (t, C-15), 73.9 (d, C-4), 88.0 (s, C-9), 101.1 (s, C-16) (*interchangeable); EIMS *m*/*z* 296 [M-$(CH_3)_2CO]^+$ (0.9), 281 [M-$(CH_3)_2CO-CH_3]^+$ (1), 221 [M-$(CH_3)_2CO-CH_3-AcOH]^+$ (31), 140 (85), 109 (59), 96 (100).

Botry-5(9)-enediol (24). The acetonide **23** (12 mg, 0.034 mmol) was dissolved in dry pyridine (2 mL) and then treated with $SOCl_2$ (100 μL) for 1 h at room temperature. The mixture was diluted with H_2O and extracted with EtOAc. The organic phase was washed with an aqueous solution of $CuSO_4$ and brine, dried, and concentrated to dryness. The residue was purified by means of normal-phase HPLC with hexane/EtOAc (7:3) as solvent to afford **24** (5 mg, 0.017 mmol, 50%) as an amorphous solid: $[\alpha]_D^{26}$ −34° (*c* 0.14, $CHCl_3$); IR (dry film) ν_{max} 3414, 1717, 1254, 1216 cm-^{1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.05 (3H, d, J_{11-2} = 7.2 Hz, H-11), 1.08 (6H, s, H*−13, H*−14), 1.14 (3H, s, H*−12), 1.47 (1H, d, $J_{7\alpha-7\beta}$ = 13.2 Hz, H-7α), 1.61 (1H, m, $J_{3\alpha-3\beta}$ = 15.2 Hz, $J_{3\alpha-4}$ = 1.6 Hz, H-3α), 1.92 (1H, d, $J_{7\beta-7\alpha}$ = 13.2 Hz, H-7*β*), 1.99 (3H, s, C*H*$_3$COO−), 2.12 (1H, m, $J_{3\beta-3\alpha}$ = 15.2 Hz, $J_{3\beta-4}$ = 5.5 Hz, H-3*β*), 2.17 (1H, m, J_{1-10} = $J_{1-10'}$ = 3.5 Hz, H-1), 2.25 (1H, m, J_{2-11} = 7.2 Hz, $J_{2-3\beta}$ = 10.1 Hz, H-2), 3.39 (1H, dd, $J_{10-10'}$ = 11.0 Hz, J_{10-1} = 3.5 Hz, H$^{\#}$-10), 3.42 (1H, d, $J_{15'-15}$ = 10.6 Hz, H-15′), 3.56 (1H, d, $J_{15-15'}$ = 10.6 Hz, H-15), 3.77 (1H, dd, $J_{10'-10}$ = 11.0 Hz, $J_{10'-1}$ = 3.5 Hz, H$^{\#}$-10′), 5.42 (1H, dd, $J_{4-3\alpha}$ = 1.6 Hz, $J_{4-3\beta}$ = 5.5 Hz, H-4) (*,$^{\#}$interchangeable); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.3 (q, C-11), 21.5 (q, *C*H$_3$COO−), 24.6 (q, C-12), 27.0 (d, C-2), 29.3 (q, C-14), 30.6 (q, C-13), 31.6 (t, C-3), 43.4 (d, C-1), 43.9 (s, C-6), 50.4 (s, C-8), 51.1 (t, C-7), 64.1 (t, C-10), 64.4 (d, C-4), 69.6 (t, C-15), 125.0 (s, C*-9), 143.6 (s, C*-5) (*interchangeable); EIMS *m*/*z* 265 [M − $CH_2OH]^+$ (15), 236 [M − $AcOH]^+$ (5), 223 (100), 205 [M − CH_2OH − $AcOH]^+$ (74).

Compound 25. A solution of **22** (33 mg, 0.105 mmol) in EtOAc (5 mL) was treated with oxalic acid for 48 h, as described above for botrydial (**1**). The mixture was neutralized and extracted with EtOAc. The crude product obtained after evaporation of the solvent was purified by means of HPLC with hexane/EtOAc (13:7) to give **25**[4] (10.5 mg, 0.035 mmol, 34%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, d, J_{11-2} = 6.3 Hz, H-11), 1.05 (1H, q, $J_{3\beta-3\alpha}$ = $J_{3\beta-2}$ = $J_{3\beta-4}$ = 12.0 Hz, H-3*β*), 1.10 (3H, s, H-14), 1.23 (1H, d, $J_{7\alpha-7\beta}$ = 12.1 Hz, H-7α), 1.28 (3H, s, H-12), 1.26 (3H, s, H-13), 1.52 (1H, ddd, $J_{1-10\beta}$ = 6.8 Hz, $J_{1-10\alpha}$ = 4.9 Hz, J_{1-2} = 11.9 Hz, H-1), 1.76 (1H, m, J_{2-11} = 6.3 Hz, $J_{2-3\beta}$ = 12.0 Hz, J_{2-1} = 11.9 Hz, H-2), 1.82 (1H, d, J_{5-4} = 12.0 Hz, H-5), 1.92 (1H, d, $J_{7\beta-7\alpha}$ = 12.1 Hz, H-7*β*), 2.02 (3H, s, C*H*$_3$COO−), 2.03 (1H, m, $J_{3\alpha-3\beta}$ = 12.0 Hz, $J_{3\alpha-4}$ = 4.6 Hz, H-3α), 3.42 (1H, d, $J_{15\alpha-15\beta}$ = 10.2 Hz, H-15α), 3.67 (1H, dd, $J_{10\alpha-10\beta}$ = 12.1 Hz, $J_{10\alpha-1}$ = 4.9 Hz, H-10α), 3.90 (1H, d, $J_{15\beta-15\alpha}$ = 10.2 Hz, H-15*β*), 3.96 (1H, dd, $J_{10\beta-10\alpha}$ = 12.1 Hz, $J_{10\beta-1}$ = 6.8 Hz, H-10*β*), 5.06 (1H, dt, $J_{4-3\beta}$ = J_{4-5} = 12.0 Hz, $J_{4-3\alpha}$ = 4.6 Hz, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.2 (q, C-11), 21.5 (q, *C*H$_3$COO−), 26.5 (q, C-13), 27.2 (q, C-14), 30.4 (d, C-2), 36.2 (q, C-12), 39.6 (t, C-3), 45.9 (s, C-8), 51.9 (d, C-1), 52.5 (t, C-7), 60.2 (d, C-5), 64.1 (t, C-10), 73.1 (d, C-4), 75.3 (t, C-15), 84.3 (s, C-9).

Compound 26. A 1 M aqueous solution of K_2CO_3 (5 mL) was added to a solution of **25** (50 mg, 0.169 mmol) in methanol. The mixture was stirred and refluxed for 3 h, the methanol was removed, and the product was extracted with EtOAc (×4). The residue obtained after evaporation of the solvent was purified by normal-phase HPLC to yield **26**[4] (15 mg, 0.059 mmol, 35%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, d, J_{11-2} = 6.5 Hz, H-11), 1.10 (1H, q, $J_{3\beta-3\alpha}$ = $J_{3\beta-2}$ = $J_{3\beta-4}$ = 12.3 Hz, H-3*β*), 1.21 (1H, d, $J_{7\alpha-7\beta}$ = 12.4 Hz, H-7α), 1.23 (3H, s, H-14), 1.32 (3H, s, H*-12), 1.29 (3H, s, H*-13), 1.47 (1H, ddd, J_{1-2} = 11.5 Hz, J_{1-10} = 6.7 Hz, $J_{1-10'}$ = 4.5 Hz, H-1), 1.51 (1H, d, J_{5-4} = 9.9 Hz, H-5), 1.71 (1H, m, J_{2-1} = 11.5 Hz, J_{2-11} = 6.5 Hz, $J_{2-3\alpha}$ = 3.0 Hz, $J_{2-3\beta}$ = 12.3 Hz, H-2), 1.90 (1H, ddd, $J_{3\alpha-3\beta}$ = 12.3 Hz, $J_{3\alpha-2}$ = 3.0 Hz, $J_{3\alpha-4}$ = 4.7 Hz, H-3α), 1.91 (1H, d, $J_{7\beta-7\alpha}$ = 12.4 Hz, H-7*β*), 3.40 (1H, d, $J_{15'-15}$ = 10.2 Hz, H-15′), 3.89 (1H, d, $J_{15-15'}$ = 10.2 Hz, H-15), 3.95 (1H, ddd, $J_{4-3\alpha}$ = 4.7 Hz, $J_{4-3\beta}$ = 12.3 Hz, J_{4-5} = 9.9 Hz, H-4), 3.67 (1H, dd, $J_{10'-10}$ = 12.0 Hz, $J_{10'-1}$ = 4.5 Hz, H-10′), 3.97 (1H, dd, $J_{10-10'}$ = 12.0 Hz, J_{10-1} = 6.7 Hz, H-10) (*interchangeable); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.3 (q, C-11), 26.3 (q, C-14), 27.4 (q, C-13), 30.6 (d, C-2), 36.7 (q, C-12), 39.5 (s, C-6), 44.4 (t, C-3), 46.0 (s, C-8), 52.0 (d, C-1), 52.4 (t, C-7), 64.0 (t, C-10), 65.1 (d, C-5), 70.2 (d, C-4), 75.1 (t, C-15), 84.3 (s, C-9).

Botryoloic Acid (28). Lactone **27** (14 mg, 0.045 mmol) in methanol was stirred with a 1 N aqueous solution of K_2CO_3 (240 μL) for 12 h at room temperature. The solution was diluted with H_2O, acidified with cold 2 N HCl, and extracted with EtOAc (30 mL, ×3). The solvent was evaporated and the residue subjected to column chromatography (eluted with hexane/EtOAc, 4:6, with drops of acetic acid) to afford **28** (10 mg, 0.030 mmol, 67%) as a white solid: mp 203−205 °C; $[\alpha]_D^{25}$ +7° (*c* 1.07, $CHCl_3$); IR (dry film) ν_{max} 3390, 1728, 1470, 1246, 1025, 953 cm^{-1}; ^1H NMR (CD_3COCD_3, 400 MHz) δ 0.97 (3H, d, J_{11-2} = 6.2 Hz, H-11), 1.06 (1H, ddd, $J_{3\beta-3\alpha}$ = 12.4 Hz, $J_{3\beta-4}$ = 11.3 Hz, H-3*β*), 1.08 (3H, s, H-14 superimposed on H-3*β*), 1.09 (3H, s, H-13), 1.18 (1H, d, $J_{7\alpha-7\beta}$ = 12.8 Hz, H-7α), 1.29 (3H, s, H-12), 1.92 (1H, d, J_{5-4} = 11.3 Hz, H-5), 2.00 (1H, m, J_{2-1} = 12.3 Hz, J_{2-11} = 6.2 Hz, $J_{2-3\alpha}$ = 3.2 Hz, H-2), 2.03 (3H, s, C*H*$_3$COO−), 2.10 (1H, m, $J_{3\alpha-3\beta}$ = 12.4 Hz, $J_{3\alpha-4}$ = 4.8 Hz, H-3α), 2.50 (1H, d, J_{1-2} = 12.3 Hz, H-1), 2.58 (1H, d, $J_{7\beta-7\alpha}$ = 12.8 Hz, H-7*β*), 3.33 (1H, d, $J_{15'-15}$ = 11.5 Hz, H-15′), 3.92 (1H, d, $J_{15-15'}$ = 11.5 Hz, H-15), 5.10 (1H, dt, $J_{4-3\alpha}$ = 4.8 Hz, $J_{4-3\beta}$ = J_{4-5} = 11.3 Hz, H-4); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 21.1 (q, C-11), 21.4 (q, *C*H$_3$COO-), 22.0 (q, C-14), 28.3 (q, C-13), 30.2 (d, C-2), 36.8 (s, C-6), 36.4 (q, C-12), 39.5 (t, C-3), 50.5 (s, C-8), 53.4 (t, C-7), 61.6 (d, C-1), 65.0 (d, C-5), 67.5 (t, C-15), 73.4 (d, C-4), 88.6 (s, C-9), 174.8 (s, C-10), 170.1 (s, CH_3COO−); EIMS *m*/*z* 295 [M − H_2O − $CH_3]^+$ (0.2), 267 [M − HCOOH − $CH_3]^+$ (0.1), 253 [M − AcOH − $CH_3]^+$ (0.4), 252 [M − HCOOH − $2CH_3]^+$ (2), 235 [M − HCOOH − $CH_2OH]^+$ (14), 232 [M − HCOOH − $CH_2OH-H_2O]^+$ (6), 177 (47), 96 (100).

Cells and Culture Conditions. Human breast ductal carcinoma (Hs578T) and adenocarcinoma (MDA-MB-231), fibrosarcoma (HT-1080), glioblastoma (U87-MG), and embryonic fibroblasts (IMR-90) were obtained from the American Type Culture Collection (Rockville, MD). Human umbilical vein endothelial cells (HUVEC) were isolated from newborns.[14] Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Paisley, Scotland) containing 10% (v/v) fetal calf serum (FCS) (BioWhittaker, Walkersville, MD), 1% glutamin, 10 μg/mL insulin (Hs578T), and 1% (v/v) vitamins (HT-1080 cells) (the latter three from Life Technologies). U87-MG cells were grown in Minimum Essential Medium (MEM) with 10% FCS, 1% sodium pyruvate, and 1% nonessential amino acids (the latter two from Life Technologies). MDA-MB-231 cells were grown in Leibovitz's L-15 medium (BioWhittaker) with 10% FCS. Primary cells HUVEC were grown in DMEM containing medium 199 (Life Technologies) with 20% FCS, 10 mM HEPES, 2.5 μg/mL fungizone (the latter two from Life Technologies), 100 μg/mL heparin, and 100 μg/mL endothelial cell growth supplement (ECGS) (the latter two from Sigma, St. Louis, MO). All cell types were cultured at 37 °C with 100 IU/mL penicillin and 0.1 mg/mL

streptomycin in a humidified atmosphere of 5% (v/v) CO_2. Cell cultures were routinely checked with the Gen-Probe rapid detection system (Gen-Probe, San Diego, CA) for mycoplasma contamination.

In Vitro Cytotoxicity Assays. Cell lines in exponential growth phase were washed twice with phosphate-buffered saline (PBS), trypsinized, and resuspended in complete culture media to 4.0×10^4 cells/mL. Cells were plated at 4 or 8×10^3 cells/well of 96-well culture plates and left for adhesion overnight at 37 °C. Botrydial (**1**) and its derivatives (**2**−**16** and **18**−**28**) were dissolved in methanol and diluted to the appropriate concentration with culture medium. Unattached cells were removed with PBS, and solutions were added to the wells to attain a total volume of 100 μL. As a negative control, 10 μL of methanol was added to the corresponding blank wells. All samples were tested in duplicate. Cells were incubated for 24 h, and then 10 μL of a 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution in PBS was added to each well. After incubating at 37 °C for 4 h, the resulting formazan precipitate was dissolved in DMSO (100 μL) and the absorbance was measured at 550 nm on a Dynatech MR 5000 multiwell plate reader (Dynatech Co., Burlington, MA).

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