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BIOLOGICALLY ACTIVE SESQUITERPENOID METABOLITES FROM THE FUNGUS *BOTRYTIS CINEREA*

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Key Word Index—*Botrytis cinerea*; Hyphomycetes; metabolites; sesquiterpenoids; dihydrobotrydial.

Abstract—Five new sesquiterpenoid metabolites, botryendial, botryenalol, 10-epi-dihydrobotrydial, methyl acetyl botryenaloate and 10-dehydroxy dihydrobotrydialone, have been isolated from *Botrytis cinerea*. The structures were elucidated by extensive NMR studies of the natural products and their derivatives.

INTRODUCTION

The fungus, *Botrytis cinerea*, is well known as the source of tricyclic sesquiterpenes with the botrydial skeleton [1–12]. Recently, we described four new metabolites from a shake culture of a strain of *B. cinerea* that had been found on grapes in a Domecq vineyard, Jerez de la Frontera, Cádiz [13]. In our previous studies, we noted that there were unknown minor metabolites that had a synergistic effect on the phytotoxicity of the fungus [14]. In order to extend our knowledge of the metabolites of *B. cinerea*, we grew the fungus in static culture on a liquid medium (Czapek–Dox) at 25–27° for about 11 days. In this paper, we describe the isolation, structure elucidation and phytotoxicity of five new metabolites (**3**, **4**, **5**, **9**, and **13**).

RESULTS AND DISCUSSION

B. cinerea (UCA 992) was cultured on Czapek–Dox medium [13]. The fermentation broth was extracted and separated into the neutral and the acidic fraction.

The neutral extract was purified using normal phase HPLC. This led to the isolation of seven products: botrydial (**1**), botrydienal (**2**) and the new natural products botryendial (**3**), botryenalol (**4**) and 10-epi-dihydrobotrydial (**5**), in addition to dihydrobotrydial (**6**) and 4β-acetoxy-9β-10β-15α-trihydroxyprobotrydial (**7**). It is interesting to note that this compound possesses the 10–15 stereochemistry for the glycol predicted [9] in biosynthetic studies on botrydial. Five products were isolated from the acid fraction, after methylation. They were the methyl ester of acetylbotryaloic acid (**8**), the new natural product methyl acetylbotryenaloate (**9**), α-*o*-methylbotrydialone (**11**), β-*o*-methylbotrydialone (**12**) and 10-dehydroxydihydrobotrydialone (**13**) which has been isolated for the first time as a natural product.

Botryendial (**3**)

The ^{13}C NMR spectrum of this compound contained 17 signals and the high-resolution mass spectrum contained an ion peak at *m/z* 232.1471 $[M - AcOH]^+$ consistent with the molecular formula $C_{17}H_{24}O_4$. An IR absorption band at 1681 cm^{-1} together with the ^{13}C NMR signals at δ_C 138.1 (*s*), 161.2 (*s*) and 191.4 (*d*) indicated that **3** was an α,β-unsaturated aldehyde. The remaining 14 carbons were assigned to a botrydial-type of sesquiterpenoid on the basis of the spectroscopic features of **3**, which were closely related to those of botrydial (**1**). In particular, the ^{1}H NMR spectrum of botryendial (**3**) was very similar to that of botrydial (**1**) except for the absence of the signal characteristic of H-1 and the significant downfield shift of the H-2 signal to δ_H 2.96 (1H, *m*, $J_{2-5} = 3.5$, $J_{2-3\beta} = 13.2$ and $J_{2-11} = 6.8$ Hz). This suggested that compound **3** was deshydroxy derivative of botrydial (**1**). The structure was confirmed by elimination of the C-9 hydroxyl group in **1**. Thus when botrydial (**1**) was refluxed in aqueous oxalic acid, two compounds were obtained whose spectroscopic data were identical to **3** and to the natural product botrydienal (**2**).

Alcohol **4**

The molecular formula, $C_{17}H_{26}O_4$, for this compound was deduced from the mass spectrum (*m/z* 294) and from the ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum of **4** was very similar to that of botrydienal (**3**) except for the absence of an aldehyde signal and the presence of two signals at δ_H 3.59 and 3.67 (*d*, each 1H,

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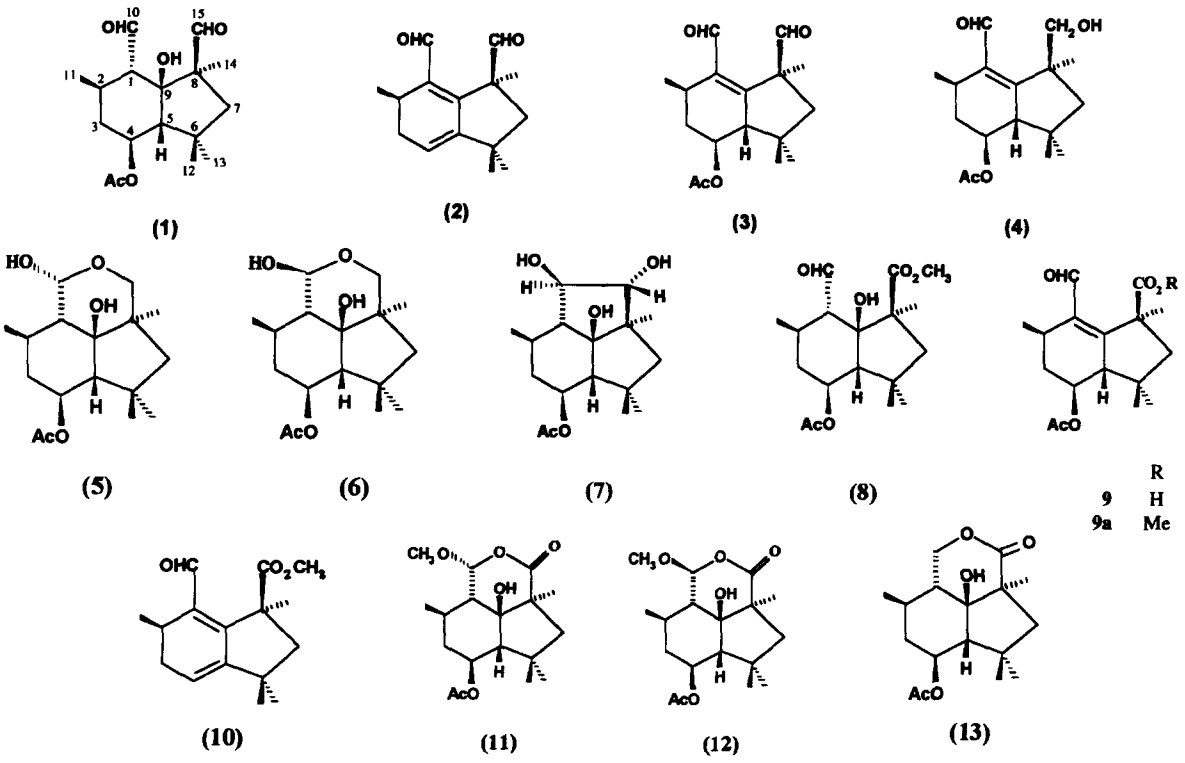

$J = 10.5$ Hz) which were correlated by COSY. This data, together with an IR absorption band at 3426 cm^{-1} indicated a change in the oxidation level of C-15 from a aldehyde to an alcohol. This was corroborated by the ^{13}C NMR spectrum which lacked an aldehyde signal and had gained instead a signal at δ_C 70.34 (*t*). This signal was correlated with the signals at δ_H 3.59 and 3.67 by a HETCOR experiment. The alcohol **4** has not been isolated previously from a *Botrytis* sp.

Compound **5**

This compound showed spectroscopic data that were very similar to those of dihydrobotrydial (**6**). The high-resolution mass spectrum indicated a molecular formula, $C_{17}H_{28}O_5$. The ^1H NMR spectrum of **5** showed the characteristic signals of a dihydrobotrydial derivative. However, the upfield shift of the signal corresponding to H-15 (from δ_H 4.17 in **6** to δ_H 3.91 in **5**) and the deshielding of the signal at δ_H 1.82 (H-2) suggested that this compound was the C-10 epimer of dihydrobotrydial (**6**). NOE experiments confirmed the stereochemistry of the hydroxyl group at C-10. In particular, irradiation of the H-10 signal caused enhancement of the H-15β, H-1 and H-11 signals while irradiation of the H-11 signal produced enhancement of the H-10, H-3α and H-1 signals. Irradiation of the H-15β signal enhanced the H-10, H-15α and C_9-OH signals thus supporting the proposed structure and stereochemistry for 10-epi-dihydrobotrydial (**5**).

Compound **9**

The high-resolution mass spectrum and ^{13}C NMR spectrum (18 signals) indicated that this compound had the molecular formula $C_{18}H_{26}O_5$. Analysis of the ^{13}C NMR spectrum by a DEPT experiment revealed signals for six methyls (δ_C 20.1, 21.3, 23.7, 29.5 (two carbons) and 52.8), two methylenes (δ_C 37.0 and 55.8), four methines (δ_C 29.3, 58.4, 70.5, 191.4) and six quaternary carbons (δ_C 39.6, 50.9, 137.4, 162.8, 170.2 and 176.5). The presence of ^1H NMR signals at δ_H 3.72 corresponding to a methoxyl group, and at δ_H 9.86 characteristic of an aldehyde group, indicated that compound **9** had a structure that was very similar to the methyl ester of botryaloic acid (**8**), a natural product that had been isolated previously from *Botrytis* sp. and which we have found in the strain under investigation. The ^{13}C NMR spectrum contained two signals at δ_C 137.4 and 162.8, corresponding to the double bond of an α,β-unsaturated aldehyde. The spectrum lacked the signal at δ_C 88.08 characteristic of C-9-OH. The ^1H NMR signals at δ_H 1.06 (H-11), 2.74 (H-5) and 2.91 (H-2) were deshielded, indicating that the compound was the dehydroxy derivative of **8**. Compound **8** was converted into compound **9** (48%) upon treatment with 6% aqueous oxalic acid solution at 100°, confirming the proposed structure. Compound **10** (50%) was also obtained from the reaction with oxalic acid.

Lactone (**13**)

Analysis of the ^{13}C NMR spectrum by a DEPT experiment revealed the presence of 17 carbons: five methyls,

three methylenes, four methines and five quaternary carbons atoms. The mass spectrum showed a $[M+1]^+$ peak (*m/z* 311) consistent with the molecular formula $C_{17}H_{26}O_5$. The ^{1}H NMR spectrum was similar to that of dihydrobotrydial (**6**). The major differences were the presence of two double-doublets at δ_H 4.25 and 4.74 (J = 12.4 Hz) and the absence of signals corresponding to the proton and hydroxyl groups at C-10. The significant downfield shift of the H-7 resonances, the IR absorption band at 1731 cm^{-1} and the $[M-CO]^+$ ion (*m/z* 222) in the mass spectrum suggested that **13** had a lactone group at C-15. This was confirmed by the presence of a signal at δ_C 173.5 in the ^{13}C NMR spectrum, which was assigned to C-15. The doublet at δ_C 92.47 corresponding to C-10 was replaced by a triplet at δ_C 70.95. The structure of compound **13** was confirmed by treatment of botrydial (**1**) with $TiCl_4/HgCl_2$ between −10 and 0°, when compound **13** was obtained; this may arise by an intramolecular Cannizaro reaction between the aldehyde groups at C-10 and C-15 followed by lactonization of the δ-hydroxy acid which is formed.

Bioassay-directed extraction and fractionation was carried out. Fractions from the fungus-free culture filtrate, from the chromatography and the purified metabolites were tested on tobacco plants. Solutions of the metabolites were prepared by dissolving the material in acetone and adding water that contained 0.1% Tween 80 to yield 1000, 500 and 250 ppm solutions. The final volume of acetone in each case was 40%. Bioassays were carried out using a methodology described previously [14]. The solutions were placed on 1 cm^{-1} diameter circles of tobacco leaves and the leaves were then incubated for a further period [14]. The results showed that compounds **5**, **7**, **8**, **9a** and **13** were inactive while **1** and **6** were active after 4 days and botrydienal (**2**) after 24 hr at 100 ppm. In addition, the dialdehyde **3** reproduced the symptoms of the plant disease after only 24 hr, when it was tested at a concentration of 250 ppm.

EXPERIMENTAL

Mp: uncorr.; ^{13}C NMR and ^{1}H NMR: 200 and 400 MHz MS: VG12-250 spectrometer at 70 eV; TLC: MN Alugran SIL G/UV 254 plates, 0.25 mm thick; CC; silica gel (Merck).

The culture of *B. cinerea* (UCA 992) employed in this work was obtained from grapes from the Domecq Vineyard, Jerez de la Frontera, Cádiz, Spain. This culture of *B. cinerea* is deposited in the Universidad de Cádiz, Facultad de Ciencias Mycological Herbarium Collection (UCA). The fungus was grown in 26 Roux bottles (200 ml) on a Czapek–Dox medium containing 0.1% yeast extract, 0.05% KH_2PO_4, 0.2% $NaNO_3$, 0.05% $MgSO_4 \cdot H_2O$, 0.01% $FeSO_4 \cdot 7H_2O$ and 5% glucose. Each bottle was inoculated with 100 μl of conidia from a suspension of 2.7×10^7 conidia/ml. The broth (5.2 l) was saturated with NaCl and acidified to pH 2 with HCl. The broth was extracted with EtOAc. The extracts were then separated into acidic and neutral fractions with aq. $NaHCO_3$ and the acidic fraction was methylated with CH_2N_2. Botrydial (**1**, 2 mg), botrydienal (**2**, 6 mg), botryendial (**3**, 1 mg), botryenalol (**4**, 1 mg), 10-epi-dihydrobotrydial (**5**, 1 mg), dihydrobotrydial (**6**, 30 mg), 4β-acetoxy-9β,10β,15α-trihydroxyprobotrydial (**7**, 3 mg), methyl acetylbotryaloate (**8**, 5 mg), methyl acetylbotryenaloate (**9**, 5 mg), α-*o*-methylbotrydialone (**11**, 0.5 mg), β-*O*-methyldihydrobotrydialone (**12**, 1 mg) and 10-dehydroxydihydrobotrydialone (**13**, 1.5 mg) were obtained from the neutral and acid fractions.

Botryendial (**3**). Oil; IR ν_{max} cm^{-1}: 2930, 2874, 1731, 1681, 1459, 1382, 1244, 1030; ^{1}H NMR ($CDCl_3$): δ 0.99 (*s*, 3H, H-13), 1.07 (*d*, 3H, J_{11-2} = 6.8 Hz, H-11), 1.21 (*s*, 3H, H-12), 1.52 (*s*, 3H, H-14), 1.38 (*m*, 1H, $J_{3\beta-4}$ = 10.3 Hz, $J_{3\beta-3\alpha}$ = 13.0 Hz, H-3β), 1.52 (*d*, 1H, $J_{7\alpha-7\beta}$ = 13.2 Hz, H-7α), 2.07 (*s*, 3H, C<u>H</u>$_3$COO), 2.14 (*d*, 1H, $J_{7\beta-7\alpha}$ = 13.2 Hz, H-7β), 2.16 (*m*, 1H, $J_{3\alpha-4}$ = 4.1 Hz, $J_{3\alpha-2}$ = 6.6 Hz, $J_{3\alpha-3\beta}$ = 13.0 Hz, H-3α), 2.57 (*dd*, 1H, J_{5-2} = 3.5 Hz, J_{5-4} = 8.9 Hz, H-5), 2.96 (*m*, 1H, J_{2-5} = 3.5 Hz, J_{2-11} = 6.8 Hz, H-2), 4.93 (*ddd*, 1H, $J_{4-3\alpha}$ = 4.1 Hz, J_{4-5} = 8.9 Hz, $J_{4-3\beta}$ = 10.3 Hz, H-4), 9.52 (*s*, 1H, H-15), 9.70 (*s*, 1H, H-10); ^{13}C NMR ($CDCl_3$): δ 20.46 (*q*, C-11), 21.29 (*q*, <u>C</u>H$_3$COO), 23.74 (*q*, C-13), 29.31 (*d*, C-2), 29.71 (*q*, C-12), 29.80 (*q*, C-14), 37.09 (*t*, C-3), 40.00 (*s*, C-6), 51.12 (*t*, C-7), 58.78 (*d*, C-5), 67.97 (*s*, C-8), 70.45 (*d*, C-4), 138.16 (*s*, C-1), 161.23 (*s*, C-9), 170.64 (*s*, CH$_3$<u>C</u>OO), 191.4 (*d*, C-10), 198.38 (*d*, C-15); MS *m/z* (rel. int.): 232 $[M-AcOH]^+$ (4), 204 $[M-AcOH-C{=}O]^+$ (100), 189 (37), 171 (27), 161 (21), 133 (19), 119 (42), 105 (23), 91 (24), 77 (15), 55 (15), 43 (73); HR-MS: obsd 232.1471 $C_{15}H_{20}O_2$ $[M-AcOH]^+$, requires 232.1463..

10-epi-*Dihydrobotrydial* (**5**). Oil; $[\alpha]_D^{20}$ +110° ($CHCl_3$, *c* 1 mg ml^{-1}); IR ν_{max} cm^{-1}: 3507, 2960, 1733, 1467, 1363, 1243, 1182, 1115, 1075; ^{1}H NMR ($CDCl_3$): δ 0.98 (*d*, 3H, J_{11-2} = 6.4 Hz, H-11), 1.10 (*s*, 3H, H-14), 1.10 (*m*, 1H, H-3β), 1.14 (*d*, 1H, $J_{7\alpha-7\beta}$ = 11.9 Hz, H-7α), 1.24 (*s*, 3H, H-13), 1.28 (*s*, 3H, H-12), 1.53 (*d*, 1H, J_{1-2} = 12.3 Hz, H-1), 1.82 (*m*, 1H, H-2), 1.85 (*d*, 1H, $J_{7\beta-7\alpha}$ = 11.9 Hz, H-7β), 1.91 (*d*, 1H, J_{5-4} = 10.6 Hz, H-5), 2.03 (*s*, 3H, C<u>H</u>$_3$COO), 2.09 (*m*, 1H, H-3α), 2.17 (*s*, 1H, OH on C-10), 3.25 (*d*, 1H, $J_{15\alpha-15\beta}$ = 10.6 Hz, H-15α), 3.35 (*s*, 1H, O<u>H</u> on C-9), 3.91 (*d*, 1H, $J_{15\beta-15\alpha}$ = 10.6 Hz, H-15β), 5.07 (*ddd*, 1H, $J_{4-3\alpha}$ = 4.6 Hz, $J_{4-3\beta}$ = 11.0 Hz, J_{4-5} = 10.6 Hz, H-4), 5.35 (*s*, 1H, H-10); ^{13}C NMR ($CDCl_3$): δ 20.07 (*q*, C-11), 21.43 (*q*, <u>C</u>H$_3$COO), 24.98 (*q*, C-14), 27.28 (*q*, C-13), 28.96 (*d*, C-2), 35.63 (*q*, C-12), 38.79 (*s*, C-6), 39.84 (*t*, C-3), 45.47 (*s*, C-8), 50.21 (*t*, C-7), 55.02 (*d*, C-1), 59.59 (*d*, C-5), 67.65 (*t*, C-15), 72.61 (*d*, C-4), 82.49 (*s*, C-9), 92.27 (*d*, C-10), 170.50 (*s*, CH$_3$<u>C</u>OO); MS *m/z* (rel. int.): 294 $[M-H_2O]^+$ (6), 276 $[M-2\times H_2O]^+$ (15), 252 $[M-AcOH]^+$ (2), 235 (12), 219 (15), 204 (22), 201 (42), 175 (29), 97 (44), 96 (50), 69 (50), 55 (100); HR-MS: obsd 294.1845 $C_{17}H_{26}O_4$ $[M-H_2O]^+$, requires 294.1831.

Botryenalol (**4**). Oil; IR ν_{max} cm^{-1}: 3426, 2927, 2866, 1735, 1671, 1460, 1375, 1246, 1031; ^{1}H NMR ($CDCl_3$): δ 0.90 (*s*, 3H, H-13), 1.05 (*d*, 3H, J_{11-2} = 6.8 Hz, H-11), 1.12 (*s*, 3H, H-12), 1.36 (*d*, 1H, $J_{3\beta-2}$ = 9.5 Hz, $J_{3\beta-4}$ = 9.4 Hz, $J_{3\beta-3\alpha}$ = 12.9 Hz, H-3β), 1.44 (*s*, 3H, H-14), 1.44 (*d*, 1H, $J_{7\alpha-7\beta}$ = 12.9 Hz, H-7α), 1.91 (*d*, 1H, $J_{7\beta-7\alpha}$ = 12.9 Hz, H-7β), 2.04 (*s*, 3H, C<u>H</u>$_3$COO), 2.03

(*ddd*, 1H, $J_{3\alpha-3\beta}$ = 12.9 Hz, $J_{3\alpha-2}$ = 6.4 Hz, $J_{3\alpha-4}$ = 4.1 Hz, H-3α superimposed on C$\underline{H}_3$COO), 2.50 (*dd*, 1H, J_{5-2} = 3.1 Hz, J_{5-4} = 8.2 Hz, H-5), 2.94 (*m*, 1H, J_{2-5} = 3.1 Hz, $J_{2-3\alpha}$ = 6.4 Hz, $J_{2-3\beta}$ = 9.5 Hz, J_{2-11} = 6.8 Hz, H-2), 3.59 (*d*, 1H, $J_{15\alpha-15\beta}$ = 10.5 Hz, H-15α), 3.67 (*d*, 1H, $J_{15\beta-15\alpha}$ = 10.5 Hz, H-15β), 4.90 (*ddd*, 1H, $J_{4-3\alpha}$ = 4.1 Hz, J_{4-5} = 8.2 Hz, $J_{4-3\beta}$ = 9.4 Hz, H-4), 10.18 (*s*, 1H, H-10); ^{13}C NMR ($CDCl_3$): δ 20.80 (*q*, C-11), 21.32 (*q*, C$\underline{H}_3$COO), 23.6 (*q*, C-13), 28.88 (*q*, C-14), 29.14 (*d*, C-2), 29.6 (*q*, C-12), 36.65 (*t*, C-3), 39.08 (*s*, C-6), 51.8 (*s*, C-8), 54.06 (*t*, C-7), 58.36 (*d*, C-5), 70.34 (*t*, C-15), 71.73 (*d*, C-4), 131.13 (*s*, C-1), 162.79 (*s*, C-9), 179.5 (*s*, CH$_3$C$\underline{O}$O), 192.4 (*d*, C-10); MS *m/z* (rel. int.): 294 $[M]^+$ (0.2), 265 $[M - CHO]^+$ (1), 234 $[M - AcOH]^+$ (4), 216 $[M - AcOH - H_2O]^+$ (5), 204 (100), 189 (23), 175 (34), 161 (17), 149 (20), 133 (20), 119 (51), 105 (21), 91 (23), 79 (16), 55 (23), 43 (84).

Methyl acetylbotryenaloate (**9**). Oil; IR ν_{max} cm^{-1}: 1737, 1681, 1245, 1143, 1092, 1050; ^{1}H NMR ($CDCl_3$): δ 0.94 (*s*, 3H, H-13), 1.06 (*d*, 3H, J_{11-2} = 6.8 Hz, H-11), 1.17 (*s*, 3H, H-12), 1.40 (*ddd*, 1H, $J_{3\beta-2}$ = 9.5 Hz, $J_{3\beta-4}$ = 10.0 Hz, $J_{3\beta-3\alpha}$ = 13.1 Hz, H-3β), 1.61 (*s*, 3H, H-14), 1.69 (*d*, 1H, $J_{7\alpha-7\beta}$ = 13.2 Hz, H-7α), 2.06 (*s*, 3H, C$\underline{H}_3$COO), 2.10 (*m*, 1H, $J_{3\alpha-4}$ = 4.2 Hz, $J_{3\alpha-2}$ = 6.5 Hz, $J_{3\alpha-3\beta}$ = 13.1 Hz, H-3α), 2.24 (*d*, 1H, $J_{7\beta-7\alpha}$ = 31.2 Hz, H-7β), 2.74 (*dd*, 1H, J_{5-2} = 3.4 Hz, J_{5-4} = 8.8 Hz, H-5), 2.91 (*m*, 1H, $J_{2-3\beta}$ = 9.5 Hz, J_{2-5} = 3.4 Hz, $J_{2-3\alpha}$ = 6.5 Hz, J_{2-11} = 6.8 Hz, H-2), 3.72 (*s*, 3H, C$\underline{H}_3$OCO), 4.92 (*ddd*, 1H, $J_{4-3\alpha}$ = 4.2 Hz, J_{4-5} = 8.8 Hz, $J_{4-3\beta}$ = 10.0 Hz, H-4), 9.86 (*s*, 1H, H-10); ^{13}C NMR ($CDCl_3$): δ 20.12 (*q*, C-11), 21.34 (*q*, C$\underline{H}_3$COO), 23.71 (*q*, C-13), 29.32 (*d*, C-2), 29.48 (*q*, C-14), 29.52 (*q*, C-12), 37.00 (*t*, C-3), 39.61 (*s*, C-6), 50.95 (*s*, C-8), 52.80 (*q*, C$\underline{H}_3$OCO), 55.80 (*t*, C-7), 58.41 (*d*, C-5), 70.51 (*d*, C-4), 137.41 (*s*, C*-1), 162.84 (*s*, C*-9), 170.22 (*s*, CH$_3$C$\underline{O}$O), 176.49 (*s*, CH$_3$O$\underline{C}$O), 191.44 (*d*, C-10); (* = interchangeable); MS *m/z* (rel. int.): 322 $[M]^+$ (0.02), 294 $[M - C{=}O]^+$ (5), 279 (4), 234 $[M - C{=}O - AcOH]^+$ (18), 219 (10), 187 (16), 175 $[M - C{=}O - AcOH - AcO]^+$ (100), 159 (33), 146 (4), 133 (13), 119 (26), 101 (10), 85 (7), 77 (6), 55 (6), 43 (20); HR-MS: obsd 322.1800, $C_{18}H_{26}O_5$, requires 322.1780.

10-Dehydroxydihydrobotridialone (**13**). Mp 153–154°; $[\alpha]_D^{25}$ + 24° ($CHCl_3$; *c* 1 mg ml^{-1}); IR ν_{max} cm^{-1}: 3444, 2967, 1731, 1474, 1468, 1248; ^{1}H NMR ($CDCl_3$): δ 0.92 (*d*, 3H, J_{11-2} = 6.4 Hz, H-11), 1.13 (*s*, 3H, H-13), 1.17 (*d*, 1H, $J_{3\beta-2}$ = 5.8 Hz, $J_{3\beta-4}$ = 11.2 Hz, $J_{3\beta-3\alpha}$ = 13.9 Hz, H-3β), 1.28 (*s*, 3H, H-12), 1.38 (*s*, 3H, H-14), 1.57 (*d*, 1H, $J_{7\alpha-7\beta}$ = 13.3 Hz, H-7α), 1.64 (*m*, 1H, $J_{2-3\alpha}$ = 3.0 Hz, $J_{2-3\beta}$ = 5.8 Hz, J_{2-1} = 12.0 Hz, J_{2-11} = 6.4 Hz, H-2), 1.94 (*m*, 1H, H-1), 1.98 (*d*, 1H, J_{5-4} = 11.3 Hz, H-5), 2.05 (*m*, 1H, $J_{3\alpha-2}$ = 3.0 Hz, $J_{3\alpha-4}$ = 4.1 Hz, $J_{3\alpha-3\beta}$ = 13.9 Hz, H-3α), 2.04 (*s*, 3H, C$\underline{H}_3$COO), 2.47 (*d*, 1H, $J_{7\beta-7\alpha}$ = 13.3 Hz, H-β), 4.25 (*dd*, 1H, $J_{10\alpha-10\beta}$ = 12.4 Hz, $J_{10\alpha-1}$ = 8.3 Hz, H-10α), 4.74 (*dd*, 1H, $J_{10\beta-10\alpha}$ = 12.4 Hz, $J_{10\beta-1}$ = 9.6 Hz, H-10β), 4.95 (*ddd*, 1H, $J_{4-3\alpha}$ = 4.1 Hz, $J_{4-3\beta}$ = 11.2 Hz, J_{4-5} = 11.3 Hz, H-4); ^{13}C NMR ($CDCl_3$): δ 19.69 (*q*, C-11), 19.98 (*q*, C-14), 21.36 (*q*, C$\underline{H}_3$COO), 27.27 (*q*, C-13), 33.75 (*d*, C-2), 36.21 (*q*, C-12), 38.47 (*t*, C-3), 39.57 (*s*, C-6), 47.89 (*d*, C-1), 49.85 (*t*, C-7), 60.96 (*d*, C-5), 70.95 (*t*, C-10), 72.58 (*d*, C-4), 86.33 (*s*, C-9), 170.36 (*s*, CH$_3$C$\underline{O}$O), 173.50 (*s*, C-15); MS *m/z* (rel. int.): 311 $[M + 1]^+$ (16), 251 (13), 250 $[M - AcOH]^+$ (31), 233 $[M + 1 - H_2O]^+$ (14), 222 $[M - C{=}O]^+$ (44), 191 (11), 175 (7), 164 (100), 149 (20), 123 (26), 111 (28), 110 (48), 95 (37), 83 (32), 69 (24), 55 (12), 43 (34).

Reaction of botrydial (**1**) *with oxalic acid.* Botrydial (**1**, 5 mg) dissolved in 6% aq oxalic acid soln (2 ml) was refluxed for 90 min [12]. The reaction mixture was neutralized with a satd soln of $NaHCO_3$ and extracted (× 3) with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed and the mixture obtained purified by normal-phase HPLC using hexane–EtOAc (17:3), yielding botryendial (**3**, 3 mg, 60%) and botrydienal (**2**, 2 mg, 40%) identical to the natural products from the fungus-free filtrate.

Reaction of methyl acetylbotryolate (**8**) *with oxalic acid* [15]. Methyl acetyl botryolate (**8**, 10 mg) was treated with oxalic acid for 30 min, as described above for botrydial (**1**). The reaction mixture was neutralized and extracted with EtOAc and the crude product obtained after evaporation of the solvent was purified by HPLC using hexane–EtOAc (3:2) yielding two products: methyl acetylbotryenaloate (**9**) (4 mg, 40%), identical to the natural product isolated from the culture, and methyl botrydienolate (**10**) (5 mg, 50%), a compound which is described for first time.

Methyl botrydienaloate (**10**). Oil; IR ν_{max} cm^{-1}: 2956, 2868, 1733, 1682, 1456, 1376, 1232, 1153; ^{1}H NMR ($CDCl_3$): δ 0.90 (*d*, 3H, J_{11-2} = 6.9 Hz, H-11), 1.08 (*s*, 3H, H-12), 1.20 (*s*, 3H, H-14), 1.35 (*d*, 1H, $J_{7\alpha-7\beta}$ = 13.4 Hz, H-7α), 1.59 (*s*, 3H, H-13), 2.15 (*m*, 1H, $J_{3\beta-4}$ = 6.2 Hz, $J_{3\beta-2}$ = 1.28 Hz, H-3β), 2.35 (*d*, 1H, *J* = 13.4 Hz, H-7β), 2.41 (*m*, 1H, H-3α), 2.93 (*m*, 1H, H-2), 3.69 (*s*, 3H, C$\underline{H}_3$OCO), 5.82 (*bdd*, 1H, $J_{4-3\beta}$ = 6.2 Hz, $J_{4-3\alpha}$ = 2.9 Hz, H-4), 9.83 (*s*, 1H, H-10); ^{13}C NMR ($CDCl_3$): δ 17.59 (*q*, C-11), 24.12 (*d*, C-2), 28.94 (*q*, C-12 and C-13), 30.34 (*q*, C-14), 30.54 (*t*, C-3), 40.34 (*s*, C-6), 51.03 (*t*, C-7), 52.54 (*q*, C$\underline{H}_3$OCO), 54.92 (*s*, C-8), 123.39 (*d*, C-4), 157.16 (*s*, C-9), 133.86 (*s*, C-1), 149.34 (*s*, C-5), 176.74 (*s*, CH$_3$O$\underline{C}$O), 190.13 (*d*, C-10); MS *m/z* (rel. int.): 248 $[M - CH_3]^+$ (7), 219 $[M - CH_3 - C{=}O]^+$ (27), 191 $[M - CH_3 - 2 \times C{=}O]^+$ (39), 173 (34), 164 (36), 149 (21), 133 (18), 121 (8), 119 (9), 93 (5), 91 (17), 77 (16), 69 (30), 55 (25), 43 (45).

Formation of 10-*dehydroxydihydrobotrydialone* (**13**) *from botrydial* (**1**) [16]. To 19 mg Mg (0.8 mmol) in THF (1 ml), 60 mg $HgCl_2$ (0.022 mmol) were added and the reaction mixture was stirred under N_2 for 15 min at room temp. The solvent was evapd and the solid obtained was washed with THF (3 × 1 ml). THF (2 ml) was then added and the reaction mixture was cooled to − 10° and 0.057 g $TiCl_4$ (0.3 mmol) in $CHCl_2$ (0.2 ml) added dropwise.

A soln of botrydial (**1**, 12 mg, 0.038 mmol) in THF (4 ml) was added to the reaction mixture which was then stirred for 90 min at 0°. Then 5 ml of satd K_2CO_3 were added and the mixture was stirred for 20 min at the same temp. The resulting mixture was filtered over Celite and the liquid washed with brine.

The organic layer was dried over $MgSO_4$ and the mixture which was obtained after the removal of the solvent was purified by CC yielding a product whose spectroscopic data were identical to those of the new natural product **12** isolated from the culture filtrate.

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