



# METABOLITES FROM A SHAKE CULTURE OF *BOTRYTIS CINerea*

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

**Abstract**—Four new metabolites, 10-oxo-dihydrobotrydial, 4β-acetoxy-9β-10β-15α-trihydroxyprobotrydial, β-O-methyldihydrobotrydialone and α-O-methyldihydrobotrydialone, were isolated from a shake culture of *Botrytis cinerea*. The second compound, a tricyclic sesquiterpene, is a key biosynthetic intermediate and sheds light on the last steps of the biosynthesis of botrydial derivatives. A higher oxidation level was observed in the metabolites isolated. The structures were elucidated by extensive NMR investigations of the natural compounds and their derivatives.

## INTRODUCTION

*Botrytis* species are serious pathogens of a number of commercial plants [1, 2]. *Botrytis cinerea* attacks a wide range of plants producing various leaf-spot diseases and grey powdery mildews on lettuces and tomatoes, and rotting of strawberries and raspberries. Two major phytotoxic metabolites are botrydial (1) and dihydrobotrydial (2). Their structures were established by a combination of chemical degradation, circular dichroism and X-ray studies [3, 4]. A series of botrydial derivatives [4–12] and some non-sesquiterpenoid metabolites [13–18] have been reported. The studies with the fungus *B. cinerea* have generally been carried out with surface cultures. In a continuation of our studies on the selective inhibition of *B. cinerea*, we undertook the study of the metabolites present in shake cultures of this fungus. In this paper, we describe the isolation and structure elucidation of four new metabolites 5–8. The isolation of a tricyclic alcohol (6) shed further light on the last steps of the biosynthesis of botrydial (1) and its relatives.

## RESULTS AND DISCUSSION

The culture of *B. cinerea* on Czapek–Dox medium was filtered to afford the mycelia and fermentation broth. This was adjusted to pH 2 with HCl solution and extracted as described in the Experimental. The neutral fraction afforded, in addition to the known compounds dihydrobotrydial (2) [3, 4] and its O-methyl and O-ethyl derivatives (3 [9] and 4 [8]), two new metabolites 5 and 6, while from the acid fraction the new metabolites 7 and 8 were isolated.

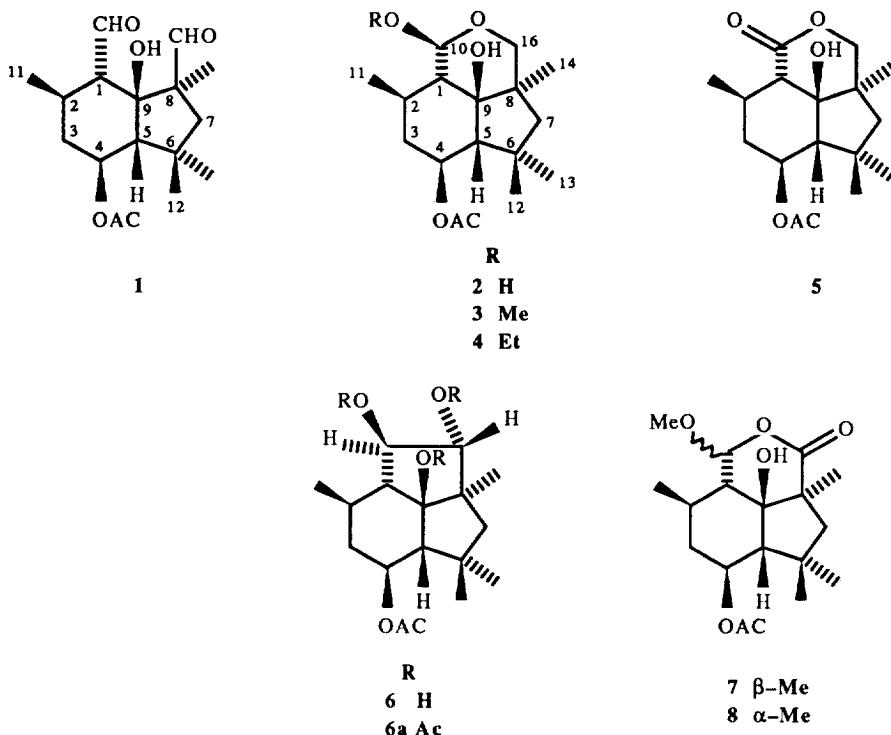
O-Ethylidihydrobotrydial (4) was isolated from the neutral fraction and was identical to a compound ob-

tained by acid treatment of 2 with ethanol, indicating that 4 was an artefact formed in the extraction process.

Compound 5 was isolated from the neutral fraction as a crystalline material which showed  $[M + 1]^+$  peak at *m/z* 311 and gave rise to a  $^{13}\text{C}$  NMR spectrum consistent with the molecular formula  $\text{C}_{17}\text{H}_{26}\text{O}_5$ . The  $^1\text{H}$  NMR spectrum was close to that of 2, but the absence of the characteristic broad singlet of H-10 and the appearance of a signal at  $\delta$  174.0 in the  $^{13}\text{C}$  NMR spectrum showed that 5 was a  $\delta$ -lactone. The shift observed in the signals of protons H-15, H-1 and H-11 were consistent with the formula proposed. The structure was confirmed by oxidation of dihydrobotrydial (2) with chromium trioxide [6–8] to yield a compound whose spectral data were identical to 5. This compound has never been isolated from *Botrytis* spp. although it has been synthesized from 2 [3, 6–8].

The tricyclic triol (6) was obtained as a yellow oil. The molecular formula  $\text{C}_{17}\text{H}_{28}\text{O}_5$  was confirmed by high resolution mass spectrometry and the  $^{13}\text{C}$  NMR spectral data. The IR showed bands for acetate groups ( $1715, 1247$  and  $1094\text{ cm}^{-1}$ ) and hydroxyl groups ( $3430\text{ cm}^{-1}$ ). The  $^{13}\text{C}$  NMR spectrum of 6 exhibited signals for five methyls ( $\delta$  20.9, 21.4, 27.5, 33.5 and 36.4), two methylenes ( $\delta$  39.6 and 48.5), six methines ( $\delta$  22.2, 58.1, 59.7, 72.7, 84.1 and 89.1) and four quaternary carbons ( $\delta$  46.6, 57.2, 95.9 and 170.5). These spectral data suggested that 6 was a saturated tricarbocyclic sesquiterpenoid possessing a tertiary and two secondary hydroxyl groups, and a secondary acetoxy group. Its  $^1\text{H}$  NMR spectrum showed two signals (*ddd*) at  $\delta$  1.95 and 5.09 and four signals corresponding to four methyl groups at  $\delta$  1.02 (*d*), 1.16 (*s*), 1.17 (*s*) and 1.29 (*s*); characteristic of a compound with a botrydiane skeleton. The signals at  $\delta$  4.43 (*d*, 1H) and 4.12 (*dd*, 1H) were assigned to protons H-15 and H-10, both geminal to hydroxyl groups, respectively. On acetylation

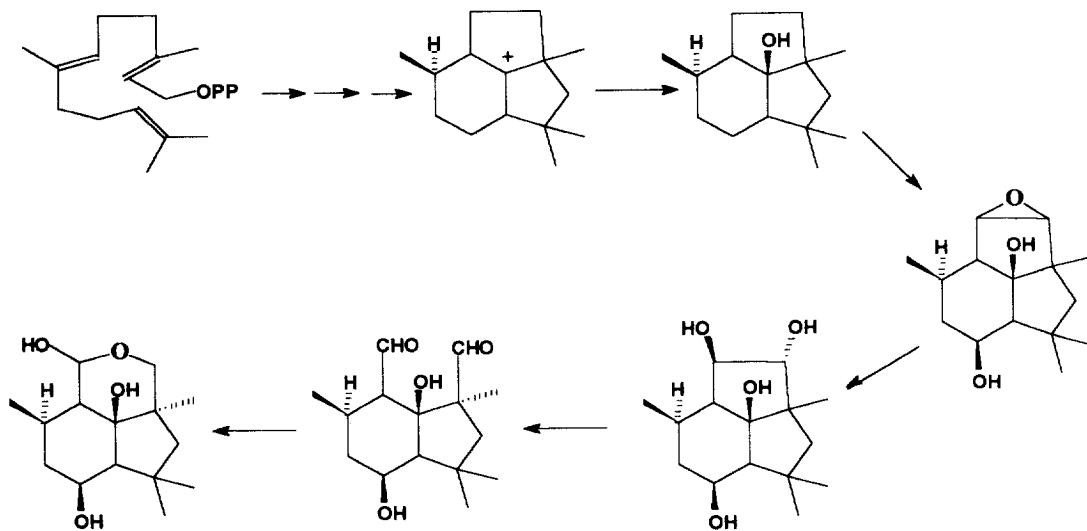
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with acetic anhydride-*p*-toluenesulphonic acid, **6** formed a tetraacetate (**6a**) with new acetate signals at  $\delta$  2.01, 2.06 and 2.10). Its  $^1\text{H}$  NMR spectrum showed that signals of H-10 and H-15 were shifted at  $\delta$  5.57 and 5.34, respectively. This tetraacetate lacked any hydroxy absorption in the IR spectrum.

The proposed structure was supported by homo- and heteronuclear-2D-correlation experiments. A 2D  $^1\text{H}$ - $[^1\text{H}]$ -shift-correlation determined the sequence of the carbon atoms through the network of  $^1\text{H}$ -spin-spin couplings, and suggested the presence of fragments C-1 (H) C-2 (H) C-3 (2H) C-4 (H) C-5 (H) and C-1 (H) C-10 (H) C-15 (H), in addition to a geminal C-7 (2H).

The orientation of hydroxyl groups at C-10 and C-15 was inferred on the basis of NOE experiments. Irradiation of the signal at  $\delta$  4.12 (H-10) led to the enhancement of those at  $\delta$  1.73 (H-2), while irradiation at  $\delta$  1.17 (H-14) enhanced the signals at  $\delta$  1.73 (H-2) and 4.12 (H-10). When the signal at  $\delta$  4.43 (H-15) was irradiated no enhancements were observed. These results indicated that H-10 and H-15 were  $\alpha$ - and  $\beta$ -orientated, respectively. The stereochemistry R (C-10), R (C-15) was supported by study of the coupling constants exhibited for signals H-1, H-10 and H-15. Thus the observed  $J_{1,10} = 2.9$  Hz and  $J_{10,15} = 5.2$  Hz were consistent with structure **6**. This compound is a key biosynthetic intermediate proposed



Scheme 1.

by Hanson [12], and clarifies the last steps of the metabolic route to botrydial from farnesyl pyrophosphate [12]. A proposal for the last steps in botrydial biosynthesis is presented in Scheme 1. The proposed epoxy intermediate is supported on the basis of the biogenetic results obtained by our group (unpublished results).

From the acid fraction was obtained a mixture of two compounds whose <sup>1</sup>H NMR spectra were very similar. The mixture was separated by HPLC yielding **7** and **8**. Both compounds showed IR absorption bands of carbonyl and acetyl groups. The mass and <sup>13</sup>C NMR spectra indicated the common molecular formula C<sub>18</sub>H<sub>28</sub>O<sub>6</sub>. The <sup>1</sup>H NMR spectrum of **7** showed a characteristic signals pattern of a dihydrobotrydial derivative with a methoxy group ( $\delta$ 3.60). The signals corresponding to the H-15 protons were absent and the <sup>13</sup>C NMR showed the presence of a carbonyl group ( $\delta$ 173.9). From these spectral data, structure **7** was inferred. The deacetyl derivative of **7** has been isolated from *B. squamosa* [9] and its reported spectral data were consistent with **7**. The structure of **8** was deduced from a comparative study of the <sup>1</sup>H NMR spectra of **7** and **8**. Both spectra were very similar but the higher coupling constant of the signal  $\delta$ 5.47 (*d*,  $J_{1,10}$  = 5.5 Hz, H-10) and the deshielded signals at  $\delta$ 2.01 (H-2), 5.47 (H-10) and 1.51 (H-14), in **8**, showed that this compound was the C-10 epimer of **7**. The stereochemistry at C-10 was confirmed by NOE experiments. Irradiation of H-10 ( $\delta$ 5.47) resulted in NOE enhancement of the signal at  $\delta$ 2.09 (H-1) indicating a  $\beta$  orientation of H-10.

This study revealed a higher oxidation level in the metabolites isolated which would be in accord with the higher rate of oxygenation in shake culture.

## EXPERIMENTAL

Mp: uncorr.; NMR: 400 MHz (<sup>1</sup>H) and 200 MHz (<sup>13</sup>C); MS: 70 eV; HPLC: Hitachi L-6270 apparatus equipped with a UV-VIS detector (L4250) and a differential refractometer detector (RI-71); TLC: MN Alugran SIL G/UV 254 plates, 0.25 mm thick; CC: Silica gel (Merck).

The culture of *Botrytis cinerea* (UCA 992) employed in this work, was obtained from grapes from the Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. A culture of this strain is deposited in the Universidad de Cádiz, Facultad de Ciencias Mycological Herbarium Collection (UCA). The fungus was grown in 40 flasks (500 ml) in an orbital shaker (200 rpm) on a Czapek-Dox medium (200 ml/flask) containing 0.1% yeast extract and 5% glucose. The broth (8 l) was satd with NaCl and acidified to pH 2 with HCl. The broth was extracted with EtOAc. The extracts were then sepd into acidic and neutral fractions with aq. NaHCO<sub>3</sub>. Dihydrobotrydial (**2**) (17 mg), *O*-methylidihydrobotrydial (**3**) (3 mg), *O*-ethyldihydrobotrydial (**4**) (8 mg), 10-oxo-dihydrobotrydial (**5**) (4 mg), 4- $\beta$ -acetoxy-9 $\beta$ -10 $\beta$ -15 $\alpha$ -trihydroxyprobotrydial (**6**) (3.2 mg),  $\beta$ -*O*-methylidihydrobotrydialone (**7**) (3 mg) and  $\alpha$ -*O*-methylidihydrobotrydialone (**8**) (1.5 mg) were obtained from the neutral and acid fractions.

*O*-Ethyldihydrobotrydial (**4**). Mp 91–93°;  $[\alpha]_D^{20}$  + 55° (CHCl<sub>3</sub>; *c* 1); IR  $\nu_{max}^{CHCl_3}$  cm<sup>-1</sup>: 3380, 2924, 2857, 1724, 1470, 1246, 1146; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$ 0.95 (*d*, 3H,  $J_{11-2}$  = 6.3 Hz, H-11), 1.04 (*m*, 1H, H-3 $\alpha$ ), 1.10 (*s*, 3H, H-14), 1.10 (*d*, 1H,  $J_{7\alpha-7\beta}$  = 11.5 Hz, H-7 $\alpha$ ), 1.21 (*t*, 3H,  $J_{2-1}$  = 7.1 Hz, H-2), 1.24 (*s*, 3H, H<sup>13</sup>-13), 1.28 (*s*, 3H, H<sup>12</sup>-12), 1.59 (*d*, 1H,  $J_{1-2}$  = 12.7 Hz, H-1), 1.82 (*m*, 1H, H-2), 1.85 (*d*, 1H,  $J_{7\beta-7\alpha}$  = 11.5 Hz, H-7 $\beta$ ), 1.90 (*d*, 1H,  $J_{5-4}$  = 11.0 Hz, H-5), 2.01 (*s*, 3H, Me-CO<sub>2</sub>), 2.03 (*m*, 1H, H-3 $\beta$ ), 3.19 (*d*, 1H,  $J_{15\alpha-15\beta}$  = 10.6 Hz, H-15 $\alpha$ ), 3.43 and 3.79 (*dc*, 2H,  $J_{1'-2'}$  = 7.1 Hz,  $J_{1'-1'}$  = 9.8 Hz, H-1'), 3.97 (*d*, 1H,  $J_{15\beta-15\alpha}$  = 10.6 Hz, H-15 $\beta$ ), 4.29 (*s*, 1H, OH), 4.92 (*s*, 1H, H-10), 5.08 (*ddd*, 1H,  $J_{4-3\beta}$  = 4.6 Hz,  $J_{4-3\alpha}$  = 11.0 Hz,  $J_{4-5}$  = 11.0 Hz, H-4); (\*interchangeable); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$ 15.1 (*c*, C-2'), 20.0 (*c*, C-11), 21.5 (*c*, Me-CO<sub>2</sub>), 25.3 (*c*, C-14), 27.3 (*c*, C-13), 28.5 (*d*, C-2), 35.6 (*c*, C-12), 38.8 (*s*, C-6), 40.0 (*t*, C-3), 45.5 (*s*, C-8), 50.4 (*t*, C-7), 54.7 (*d*, C-1), 59.4 (*d*, C-5), 63.0 (*t*, C-1'), 67.5 (*t*, C-15), 72.8 (*d*, C-4), 82.6 (*s*, C-9), 97.1 (*d*, C-10), 170.6 (*s*, Me-CO<sub>2</sub>); MS *m/z* (rel. int.): 341 [M + 1]<sup>+</sup> (0.1), 281 [M + 1 – HOAc]<sup>+</sup> (0.5), 149 (9), 83 (27), 69 (34), 57 (38), 55 (62), 43 (100).

*Reaction of dihydrobotrydial (**2**) with HCl/EtOH.* A soln of dihydrobotrydial (10 mg) in EtOAc, was treated with 2M HCl (2 ml) and EtOH (1 ml). The reaction mixture was stirred for 2 hr, then neutralized and extracted with EtOAc (3  $\times$  15 ml). The solvent was evapd and the crude extract chromatographed to yield *O*-ethyldihydrobotrydial (**4**) (8 mg).

*10-Oxodihydrobotrydial (**5**).* Mp 207°;  $[\alpha]_D^{20}$  + 9° (CHCl<sub>3</sub>; *c* 1); IR  $\nu_{max}$  cm<sup>-1</sup>: 3482, 2918, 2852, 1707, 1243, 1080; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$ 1.12 (*m*, 1H, H-3 $\alpha$ ), 1.13 (*s*, 3H, H-14), 1.26 (*s*, 3H, H<sup>13</sup>-13), 1.27 (*d*, 3H,  $J_{11-2}$  = 5.1 Hz, H-11), 1.29 (*s*, 3H, H<sup>12</sup>-12), 1.33 (*d*, 1H,  $J_{7\alpha-7\beta}$  = 12.5 Hz, H-7 $\alpha$ ), 1.86 (*d*, 1H,  $J_{5-4}$  = 11.0 Hz, H-5), 1.90 (*d*, 1H,  $J_{7\beta-7\alpha}$  = 12.5 Hz, H-7 $\beta$ ), 1.97 (*m*, 1H, H-2), 2.03 (*s*, 3H, Me-CO<sub>2</sub>), 2.09 (*ddd*, 1H,  $J_{3\beta-2}$  = 2.9 Hz,  $J_{3\beta-4}$  = 4.1 Hz,  $J_{3\beta-3\alpha}$  = 11.0 Hz, H-3 $\beta$ ), 2.26 (*d*, 1H,  $J_{1-2}$  = 11.7 Hz, H-1), 3.92 (*d*, 1H,  $J_{15\alpha-15\beta}$  = 9.8 Hz, H-15 $\alpha$ ), 4.69 (*d*, 1H,  $J_{15\beta-15\alpha}$  = 9.8 Hz, H-15 $\beta$ ), 5.02 (*ddd*, 1H,  $J_{4-3\beta}$  = 4.1 Hz,  $J_{4-3\alpha}$  = 11.0 Hz,  $J_{4-5}$  = 11.0 Hz, H-4); (\*interchangeable); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$ 22.0 (*c*, Me-CO<sub>2</sub>), 22.3 (*c*, C-11), 23.9 (*c*, C-14), 27.7 (*c*, C-13), 32.1 (*d*, C-2), 36.9 (*c*, C-12), 40.7 (*t*, C-3), 41.5 (*s*, C-6), 44.5 (*s*, C-8), 49.9 (*t*, C-7), 58.2 (*d*, C-1), 62.2 (*d*, C-5), 72.3 (*d*, C-4), 76.7 (*t*, C-15), 84.8 (*s*, C-9), 169.1 (*s*, Me-CO<sub>2</sub>), 174.0 (*s*, C-10); MS *m/z* (rel. int.): 311 [M + 1]<sup>+</sup> (10), 251 [M + 1 – HOAc]<sup>+</sup> (8), 233 [M + 1 – HOAc-H<sub>2</sub>O]<sup>+</sup> (15), 177 (19), 149 (19), 96 (33), 43 (100); HR-MS: obsd. 310.1780 [M]<sup>+</sup>, requires 310.17802.

*4 $\beta$ -Acetoxy-9 $\beta$ ,10 $\beta$ ,15 $\alpha$ -trihydroxyprobotrydial (**6**).* Yellow oil; IR  $\nu_{max}$  cm<sup>-1</sup>: 3430, 2925, 2856, 1715, 1468, 1247, 1094; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$ 1.02 (*d*, 3H,  $J_{11-2}$  = 6.4 Hz, H-11), 1.10 (*m*, 1H, H-3 $\alpha$ ), 1.16 (*s*, 3H, H-13), 1.17 (*s*, 3H, H-14), 1.20 (*d*, 1H,  $J_{7\alpha-7\beta}$  = 11.7 Hz, H-7 $\alpha$ ), 1.25 (*m*, 1H, H-1), 1.29 (*s*, 3H, H-12), 1.70 (*d*, 1H,  $J_{5-4}$  = 10.7 Hz, H-5), 1.73 (*m*, 1H, H-2), 1.95 (*ddd*, 1H,  $J_{3\beta-2}$  = 3.4 Hz,  $J_{3\beta-4}$  = 3.8 Hz,  $J_{3\beta-3\alpha}$  = 10.7 Hz, H-3 $\beta$ ), 2.02 (*s*, 3H, Me-CO<sub>2</sub>), 4.12 (*dd*, 1H,  $J_{10-1}$  = 2.9 Hz,  $J_{10-15}$  = 5.2 Hz, H-10), 4.43 (*d*, 1H,  $J_{15-10}$  = 5.2 Hz, H-15), 5.09 (*ddd*, 1H,  $J_{4-3\beta}$

$\delta = 3.8$  Hz,  $J_{4-3\alpha} = 10.7$  Hz,  $J_{4-5} = 10.7$  Hz, H-4);  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>):  $\delta$  20.86 (c, C-11), 21.4 (c, Me-CO<sub>2</sub>), 22.2 (d, C-2), 27.5 (c, C-13), 33.5 (c, C-14), 36.4 (s, C-12), 39.6 (t, C-3), 46.6 (s, C\*-6), 48.5 (t, C-7), 57.2 (c, C\*-8), 58.1 (d, C-1), 59.7 (d, C-5), 72.7 (d, C-4), 84.1 (d, C-15), 89.1 (d, C-10), 95.9 (s, C-9), 170.5 (s, Me-CO<sub>2</sub>); (\* = interchangeable); MS  $m/z$  (rel. int.): 252 [M - HOAc]<sup>+</sup> (2), 234 [M - HOAc-H<sub>2</sub>O]<sup>+</sup> (4), 219 (7), 205 (10), 175 (15), 109 (21), 95 (21), 55 (25), 43 (100); HR-MS: obsd. 252.1724 C<sub>15</sub>H<sub>24</sub>O<sub>3</sub> [M - MeCO<sub>2</sub>H]<sup>+</sup>, requires 252.17254.

4 $\beta$ -9 $\beta$ -10 $\beta$ -15 $\alpha$ -Tetraacyloxyprobotrydial (6a). Triol 6 (3 mg) was dissolved in Ac<sub>2</sub>O (2 ml) and a catalytic amount of *p*-toluenesulphonic acid was added. The reaction mixture was stirred for 52 hr at room temp. The soln was poured on ice, neutralized with Na<sub>2</sub>CO<sub>3</sub> and the product recovered in EtOAc. The extract was washed with brine and H<sub>2</sub>O, dried and evapd to afford 6a (2 mg). Oil; IR  $\nu_{\text{max}}$  cm<sup>-1</sup>: 2927, 1747, 1371, 1238;  $^1\text{H}$  NMR (CDCl<sub>3</sub>):  $\delta$  0.98 (d, 3H,  $J_{11-2} = 5.6$  Hz, H-11), 1.14 (s, 3H, H-13), 1.15 (s, 3H, H-14), 1.21 (s, 3H, H-12), 2.01 (s, 3H, Me-CO<sub>2</sub>), 2.02 (s, 3H, Me-CO<sub>2</sub>), 2.06 (s, 3H, Me-CO<sub>2</sub>), 2.10 (s, 3H, Me-CO<sub>2</sub>), 2.57 (d, 1H,  $J_{5-4} = 10.7$  Hz, H-5), 5.10 (ddd, 1H,  $J_{4-3\beta} = 3.8$  Hz,  $J_{4-3\alpha} = 10.7$  Hz,  $J_{4-5} = 10.7$  Hz, H-4), 5.34 (d, 1H,  $J_{15-10} = 5.5$  Hz, H-15), 5.57 (dd, 1H,  $J_{10-1} = 2.8$  Hz,  $J_{10-15} = 5.5$  Hz, H-10); MS  $m/z$  (rel. int.): 379 [M - AcO]<sup>+</sup> (0.3), 336 [M - AcO - Me - CO]<sup>+</sup> (1), 276 [M - AcO - Me - CO - HOAc]<sup>+</sup> (18), 216 [M - AcO - Me - CO - 2  $\times$  HOAc]<sup>+</sup> (75), 201 (38), 160 (89), 43 (100); HR-MS: obsd. 318.1848 C<sub>19</sub>H<sub>26</sub>O<sub>4</sub> [M - 2  $\times$  MeCO<sub>2</sub>H]<sup>+</sup>, requires 318.1831.

$\beta$ -O-Methyldihydrobotrydialone (7). Mp 52°;  $[\alpha]_D^{20} + 51^\circ$  (CHCl<sub>3</sub>; *c* 0.7); IR  $\nu_{\text{max}}$  cm<sup>-1</sup>: 3466, 2927, 1735, 1365, 1251, 1126, 1030;  $^1\text{H}$  NMR (CDCl<sub>3</sub>):  $\delta$  0.99 (d, 3H,  $J_{11-2} = 5.9$  Hz, H-11), 1.11 (s, 3H, H-14), 1.16 (m, 1H, H-3 $\alpha$ ), 1.27 (s, 3H, H\*-13), 1.29 (s, 3H, H\*-12), 1.55 (d, 1H,  $J_{7\alpha-7\beta} = 13.3$  Hz, H-7 $\alpha$ ), 1.70 (m, 1H, H-1), 1.70 (m, 1H, H-2 superimpost.), 1.97 (d, 1H,  $J_{5-4} = 11.2$  Hz, H-5), 2.03 (s, 3H, Me-CO<sub>2</sub>), 2.08 (m, 1H, H-3 $\beta$ ), 2.46 (d, 1H,  $J_{7\beta-7\alpha} = 13.3$  Hz, H-7 $\beta$ ), 3.60 (s, 3H, MeO), 4.92 (ddd, 1H,  $J_{4-3\beta} = 4.1$  Hz,  $J_{4-3\alpha} = 11.2$  Hz,  $J_{4-5} = 11.2$  Hz, H-4), 5.19 (d, 1H,  $J_{10-1} = 4.5$  Hz, H-10); (\* = interchangeable);  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>):  $\delta$  20.1 (c, C-14), 20.4 (c, C-11), 21.4 (c, Me-CO<sub>2</sub>), 27.4 (c, C-13), 32.3 (d, C-2), 36.0 (c, C-12), 38.7 (t, C-3), 40.0 (s, C-6), 49.0 (t, C-7), 56.3 (d, C-1), 57.4 (c, CH<sub>3</sub>O), 60.5 (d, C-5), 72.3 (d, C-4), 83.4 (s, C-9), 107.7 (d, C-10); MS  $m/z$  (rel. int.): 280 [M - HOAc]<sup>+</sup> (0.3), 249 [M - HOAc - MeO]<sup>+</sup> (0.4), 236 (3), 220 (6), 204 (20), 175 (25), 151 (50), 111 (25), 85 (100), 43 (87); HR-MS: obsd. 280.1694 [M - HOAc]<sup>+</sup>, requires 280.16745.

$\alpha$ -O-Methyldihydrobotrydialone (8). Mp 92-94°;  $[\alpha]_D^{20} - 10^\circ$  (CHCl<sub>3</sub>; *c* 0.7); IR  $\nu_{\text{max}}$  cm<sup>-1</sup>: 3478, 2929, 2877, 1721, 1459, 1364, 1247, 1133, 1085;  $^1\text{H}$  NMR (CDCl<sub>3</sub>):  $\delta$  0.99 (d, 3H,  $J_{11-2} = 5.9$  Hz, H-11), 1.10 (m, 1H, H-3 $\alpha$ ), 1.13 (s, 3H, H\*-13), 1.25 (s, 3H, H\*-12), 1.51 (s, 3H, H-14), 1.61 (d, 1H,  $J_{7\alpha-7\beta} = 13.0$  Hz, H-7 $\alpha$ ), 1.93 (d, 1H,  $J_{5-4} = 11.2$  Hz, H-5), 2.01 (m, 1H, H-2), 2.03 (s, 3H, Me-CO<sub>2</sub>), 2.09 (d, 1H,  $J_{1-10} = 7.8$  Hz, H-1), 2.09 (m, 1H, H-3 $\beta$ , H-1),

2.39 (d, 1H,  $J_{7\beta-7\alpha} = 13.0$  Hz, H-7 $\beta$ ), 3.53 (s, 3H, MeO), 4.93 (ddd, 1H,  $J_{4-3\beta} = 3.9$  Hz,  $J_{4-3\alpha} = 11.2$  Hz,  $J_{4-5} = 11.2$  Hz, H-4), 5.47 (d, 1H,  $J_{10-1} = 7.8$  Hz, H-10); (\* = interchangeable);  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>): 20.6 (c, Me-CO<sub>2</sub>), 21.3 (c, C-11), 27.3 (c, C-13), 29.3 (c, C-14), 31.2 (d, C-2), 36.2 (c, C-12), 39.0 (t, C-3), 39.2 (s, C-6), 50.1 (t, C-7), 53.2 (d, C-1), 54.2 (s, C-8), 57.3 (c, MeO), 61.4 (d, C-5), 72.3 (d, C-4), 87.2 (s, C-9), 103.6 (d, C-10), 172.0 (s, Me-CO<sub>2</sub>), 174.3 (s, C-15); MS  $m/z$  (rel. int.): 341 [M + 1]<sup>+</sup> (0.5), 281 [M + 1 - HOAc]<sup>+</sup> (2), 263 (12), 236 (34), 204 (100), 151 (85), 85 (72); HR-MS: obsd. 280.1660 [M - OHAc]<sup>+</sup>, requires 280.16745.

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