Journal of Chemical Ecology, Vol. 20, No. 10, 1994

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF ANALOGUES OF NATURALLY OCCURRING BOTRYDIAL PRECURSORS

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(Received April 7, 1994; accepted May 31, 1994)

Abstract—Analog compounds of the proposed intermediates of the biogenetic pathway to botrydial have been synthesized. These compounds were tested for their potential antifungal activity against the phytopathogen *Botrytis cinerea*. Our results showed a fungistatic effect of some compounds on mycelium growth. The most significant effect was exerted by 2-α-hydroxy-2,3-dihydro-1-epiprobotrydial, which inhibited growth of *B. cinerea*. Some aspects of structure-activity relationships are discussed.

Key Words—Synthesis, antifungal, *Botrytis cinerea*, bioassay, structure-activity.

INTRODUCTION

Botrytis species are serious pathogens of a number of commercial plants (Whealer, 1969; Coley-Smith et al., 1980). *Botrytis cinerea* attacks a wide range of plants, causing several leaf-spot diseases and grey powdery mildews on lettuces and tomatoes, and rotting of strawberries and raspberries. *Botrytis alli*, *Botrytis byssoidea*, and *Botrytis squamosa* cause neck rots of onions, and *Botrytis tulipae* and *Botrytis fabae* produce a leaf-spot disease on beans, which can result in substantial crop losses (Coley-Smith et al., 1980). The rapid development of tolerance of *Botrytis* spp. to the commercial fungicides has led to an

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0098-0331/94/1000-2631$07.00/0

increase in the amount of these compounds used, with the subsequent additional problems of persistence in the ecosphere and its incorporation to the food chain. Since 1968, knowledge of *Botrytis cinerea* metabolism has increased quite rapidly. Several botrydial derivatives (Fehlhaber et al., 1974; Lindner and Gross, 1974; Cuevas and Hanson, 1977; Bradshaw and Hanson, 1980; Kimura et al., 1986; Kimata et al. 1985) and some nonsesquiterpenoids metabolites have also been detected (Overeem and Van Dijkman, 1968; Arpin et al., 1977; Welmar et al., 1979; Suga et al., 1984; Cutler et al., 1988).

Botrydial and structurally related compounds are characteristic metabolites of *Botrytis* species. Botrydial (Figure 1, **1**) is a bicyclic nonisoprenoid sesquiterpene, which was isolated from culture of the fungus *Botrytis cinerea* (Fehlhaber et al., 1974). The role of these molecules in fungus physiology is unknown. We have undertaken the synthesis of analogs of botrydial precursors and the study of their fungicidal activity in order to enhance our knowledge of these sesquiterpenes biosynthesized by *B. cinerea*.

METHODS AND MATERIALS

General Methods. Melting points were measured with a Kofler block Reichert-Jung apparatus and are uncorrected. ^{13}C and ^{1}H NMR spectra were recorded with a Varian Gemini 200 and Unity 400, IR spectra with a Perkin Elmer 881 instrument, and mass spectra were recorded on a VG12-250 spectrometer at 70 eV. Optical rotations were determined with a Perkin Elmer 241 polarimeter. HPLC was performed with a Hitachi L-6270 apparatus equipped with a UV-VIS detector (L 4250) and a differential refractometer detector (RI-71). TLC was performed on MN Alugran SIL G/UV 254 plates, 0.25 mm thick. Silica gel (Merck) was used for column chromatography.

Microorganism and Antifungal Assay. The culture of *Botrytis cinerea* employed in this work, *Botrytis cinerea* (UCA 992), was obtained from grapes

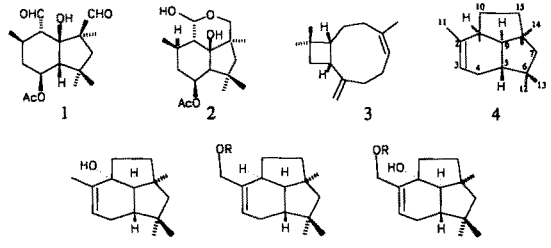


FIG. 1.

of the Domecq vineyard, Jerez de la Frontera, Cádiz, Spain. The culture of *Botrytis cinerea* is deposited in the Universidad de Cádiz, Facultad de Ciencias Mycological Herbarium Collection (UCA). The synthesized compounds were tested on *Botrytis cinerea* by the "poisoned food technique." Chemicals were solubilized in ethanol and incorporated into the culture medium (glucose, malt, peptone, agar) at a concentration of 10–300 ppm. The final alcohol concentration was identical in controls and treated culture. A 5-mm-diameter mycelial disk of an actively growing culture of *Botrytis cinerea* was placed in the center of a 6-cm-diameter agar plate. The diameter of the fungal colony was measured daily for seven days. Percent growth inhibition over control was calculated by following the method previously described (Patil et al., 1986).

Epoxidation of 1-Epiprobotrydial ***(4).*** See Gollnick and Shade (1970) and Collado et al. (1994). A solution of **4** (351 mg) in 2 ml of Et_2O was treated with 592 mg of MCPBA in 10 ml of Et_2O. The reaction mixture was stirred at 0°C for 7 hr. Then the solvent was removed and the crude product purified by column chromatography (CC) and HPLC (hexane–AcOEt 99 : 1) yielding 19 mg of unreacted starting material **(4),** 132 mg of 2β,3β-epoxy-1-epiprobotrydial (Figure 2, **17,** 35%) and 212 mg of 2α,3α-epoxy-1-epiprobotrydial (**16,** 56%). 2β,3β-epoxy-1-epiprobotrydial **(17),** colorless oil; ν^{film} 2972, 2884, 1437, 1363, 1298, 1256, 1193, 1081, 832, 711 cm^{-1}. ^1H NMR: 0.82 (s, 3H, H-13), 0.95 (s, 3H, H-12), 1.03 (s, 3H, H-14), 1.33 (s, 3H, H-11), 1.38 (m, 1H, H-5), 1.40–1.56 (m, 4H, H-10, H-15, H-7, and H-7′), 1.62 (m, 1H, H-4), 1.80–2.00 (m, 3H, H-4′, H-10′, H-15′), 2.25 (ddd, 1H, J = 6.8, 8.2 and 9.2 Hz, H-1), 3.11 (br d, 1H, J = 2.1 Hz, H-3). ^{13}C NMR: 23.09 (q, C-13), 25.16 (q, C-11), 26.02 (t, C-15), 26.26 (t, C-4), 28.92 (q, C-12), 30.49 (q, C-14), 40.48 (s, *C-6), 41.26 (d, C-1), 42.36 (d, C-5), 42.97 (t, C-10), 46.30 (s,

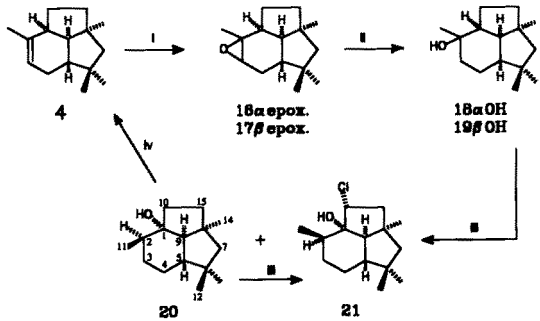


FIG. 2.

C-8), 57.13 (d, C-9), 59.02 (t, C-7), 59.71 (s, C-2), 63.44 (d, C-3) (assignments may be interchanged). Mass spectrum: *m/z* 220 (12, M^+), 205 (16), 202 (2) [M^+-H_2O], 187 (21), 161 (55), 123 (52), 121 (42), 109 (81), 107 (57), 93 (52), 91 (67), 79 (68), 77 (64), 67 (40), 55 (55), 43 (100), 41 (98). 2α,3α-epoxy-1-epiprobotrydial **(16),** mp 81–82°C; ν^{KBr} 3396, 2958, 2883, 1441, 1360, 1214, 1118, 1060, 945, 862, 812, 753 cm^{-1}. ^1H NMR: 0.75 (s, 3H, H-13), 0.94 (s, 3H, H-12), 1.03 (s, 3H, H-14), 1.18 (ddd, 1H, J_{5-9} = 12.8 Hz, $J_{5-4'}$ = 4.8 Hz and J_{5-4} = 12.8 Hz, H-5), 1.28 (s, 3H, H-11), 1.62–1.78 (m, 3H, H-10, H-10′, H-9), 1.87 (ddd, 1H, $J_{4-4'}$ = 14.0 Hz, $J_{5-4'}$ = 4.8 Hz, $J_{3-4'}$ = 5.8 Hz, H-4′), 2.49 (m, 1H, H-1), 3.08 (d, 1H, $J_{3-4'}$ = 5.8 Hz, H-3). ^{13}C NMR: 22.16 (q, C-11), 22.66 (q, C-13), 25.73 (t, C-4), 27.97 (t, C-15), 28.94 (q, C-12), 31.00 (q, C-14), 40.45 (s, *C-6), 43.74 (t, C-10), 44.24 (d, C-1), 45.84 (s, *C-8), 47.63 (d, C-5), 55.10 (d, C-9), 59.28 (t, C-7), 62.08 (s, C-2), 62.46 (d, C-3) (* assignments may be interchanged). Mass spectrum: *m/z* 220 (11, M^+), 205 (21), 202 (2, M^+-H_2O), 187 (27), 176 (20), 161 (100), 151 (37), 135 (19), 123 (27), 109 (48).

*2α-Hydroxy-2,3-dihydro-1-epiprobotrydial **(18).*** Compound **16** (22 mg), dissolved in 5 ml of Et_2O, was treated for 16 hr with 0.3 ml of $LiAlH_4$ in Et_2O (1 M). Then, H_2O (10 ml) was slowly added and the reaction mixture extracted with Et_2O. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded a crude reaction product that was purified by CC (hexane–AcOEt, 99:1) yielding **18** (19 mg, 86%) as a colorless oil, $[\alpha]_D^{25}$ −36° ($CHCl_3$, c = 2.25 10^{-3}); ν^{film} 3373, 2947, 2862, 1453, 1370, 1298, 1186, 1133, 1053, 1023, 986, 940, 918, 898, 746 cm^{-1}. ^1H NMR: 0.81 (s, 3H, H-13), 0.97 (s, 3H, H-12), 1.02 (s, 3H, H-14), 1.09 (ddd, 1H, *J* = 12.4, 12.2, and 2.5 Hz, H-5), 1.16 (s, 3H, H-11), 1.23 (dddd, 1H, *J* = 12.2, 12.4, 12.3, and 3.5 Hz, H-4), 1.36–1.44 (m, 3H, H-15, H-15′, and H-4′ overlapped), 1.56–1.70 (m, 2H, H-10′, and H-3′), 1.84 (dd, 1H, *J* = 5.5 and 12.4 Hz, H-9), 2.09 (m, 1H, H-1). ^{13}C NMR: 21.56 (t, C-4), 23.37 (q, C-13), 27.52 (t, C-15), 29.46 (q, C-12), 29.62 (q, C-11), 30.32 (q, C-14), 37.78 (t, C-10), 40.72 (s, *C-6), 43.00 (t, C-3), 46.24 (s, *C-8), 50.78 (d, C-5), 50.86 (d, C-1), 56.43 (d, C-9), 59.11 (t, C-7), 72.66 (s, C-2) (*assignments may be interchanged). Mass spectrum: *m/z* 222 (31, M^+), 207 (25), 204 (10, M^+-H_2O), 189 (33), 177 (35), 165 (42), 149 (100), 137 (39), 121 (30), 109 (70), 95 (77), 81 (52), 71 (30), 55 (17), 43 (43), 42 (6).

*2β-Hydroxy-1-epiprobotrydial **(19).*** Compound **19** was prepared following the procedure described for **18.** Thus, 78 mg of **17** afforded 41 mg (52%) of **19** and 20 mg of starting material. 2β-Hydroxy-1-epiprobotrydial **(19),** mp 90–91°C, $[\alpha]_D^{25}$ −0.7° ($CHCl_3$, c = 6.05 × 10^{-3}); ν^{KBr} 3336, 2932, 2866, 1454, 1367, 1148, 1012, 908, 762, 652. ^1H NMR: 0.78 (s, 3H, H-12), 0.97 (s, 3H, H-13), 1.01 (s, 3H, H-14), 1.00 (ddd, 1H, *J* = 12.2, 3.9, and 11.9 Hz, H-4), 1.24 (ddd, 1H, *J* = 12.4, 12.5, and 2.8 Hz, H-5), 1.30 (s, 3H, H-11), 1.44–

1.52 (m, 2H, H-3 and H-4'), 1.52–1.70 (m, H-9, H-7, and H-7'), 1.81 (m, 1H, H-10'), 2.19 (m, 1H, H-1). ^{13}C NMR: 23.24 (t, C-4), 23.29 (q, C-13), 25.93 (t, C-15), 29.52 (q, C-12), 30.25 (q, C-14), 30.67 (q, C-11), 38.46 (t, C-10), 40.31 (s, *C-6), 42.63 (t, C-3), 46.32 (s, *C-8), 50.14 (d, C-1), 50.81 (d, C-5), 58.37 (d, C-9), 59.07 (t, C-7), 73.32 (s, C-2) (* assignments may be interchanged). Mass spectrum: *m/z* 222 (12, M^+), 207 (12), 204 (5, M^+-H_2O), 189 (23), 177 (18), 165 (23), 149 (55), 137 (36), 121 (28), 109 (77), 95 (100), 81 (64), 71 (65).

Treatment of 2α-Hydroxy-1-epiprobotrydial ***(18)*** *with $FSO_3H/ClFSO_2$.* To a cold flask (−120°C), under N_2 atmosphere, 0.15 ml of FSO_3H, 0.7 ml of $ClFSO_2$ and 46 mg of **18** were added. The reaction mixture was stirred for 45 min and then 5 ml of acetone–water (5:1) was slowly added. The mixture obtained was subjected to column chromatography, yielding 16 mg of unreacted starting material **(18),** 8 mg of 2α,3-dihydro-1α-hydroxy-1-epiprobotrydial (**20,** 17%) (Khomenko et al., 1985), and 1 mg of 10α-chloro-2α,3-dihydro-1α-hydroxy-1-epiprobotrydial **(21);** mp 78–82°C, $[\alpha]_D^{25}$ −16.3° ($CHCl_3$, c = 1.6 10^{-3}), ν^{KBr} 3592, 2969, 2950, 2882, 1437, 1353, 1298, 1249, 1130, 1102, 1029, 972, 948, 883, 856, 810, 729, 685. ^{1}H NMR: 0.79 (s, 3H, H-12), 0.94 (s, 3H, H-13), 1.02 (d, 3H, $J_{11\text{-}2}$ = 6.7 Hz, H-11), 0.95–1.15 (m, 3H, H-5, H-4, and H-3), 1.31 (s, 3H, H-14), 1.49 (s, 2H, H-7 and H-7'), 1.55 (m, 1H, H-4'), 1.75–1.85 (m, 3H, H-9, H-2, and H-3'), 2.01 (dd, 1H, $J_{10\text{-}15}$ = 8.8 Hz, $J_{15\text{-}15'}$ = 14.2 Hz, H-15), 2.36 (dd, 1H, $J_{10\text{-}15'}$ = 9.0 Hz, $J_{15\text{-}15'}$ = 14.2 Hz, H-15'), 4.59 (dd, 1H, $J_{10\text{-}15}$ = 8.8 Hz, $J_{10\text{-}15'}$ = 9.0 Hz, H-10). ^{13}C NMR: 16.43 (q, C-11), 22.13 (q, C-13), 25.05 (t, C-4), 28.82 (q, C-12), 32.60 (q, C-14), 35.08 (t, C-3), 40.75 (s, *C-6), 41.50 (d, C-9), 44.41 (s, *C-8), 54.22 (t, C-15), 55.59 (d, C-5), 59.59 (t, C-7), 62.89 (d, C-2), 63.25 (d, C-10), 81.82 (s, C-1) (* assignments may be interchanged). Mass spectrum: *m/z* 258 (M^++2), 256 (7, M^+), 243 (2), 241 (8), 221 (25, M^+-Cl), 220 (37, M^+-HCl), 200 (66), 185 (26), 164 (35), 135 (49), 119 (52), 111 (79), 109 (83), 91 (75), 77 (55), 67 (48), 55 (100).

Treatment of 2β-Hydroxy-1-epiprobotrydial ***(19)*** *with $FSO_3H/ClFSO_2$.* Compound **19** (20 mg) was subjected to the same procedure described above for **18** yielding 2 mg of 1-epiprobotrydial **(4),** 10 mg of 2α,3-dihydro-1α-hydroxy-1-epiprobotrydial (**20,** 50%), and 2 mg of 10α-chloro-2α,3-dihydro-1α-hydroxy-1-epiprobotrydial (**21,** 8%).

Treatment of 1-Epiprobotrydial ***(4)*** *with SeO_2.* Compound **4** (Gollnick and Shade, 1970; Collado et al., 1994) (135 mg) dissolved in 10 ml of dry CH_2Cl_2, was treated with 0.25 ml of *t*-BuOOH (80%) and 52 mg of SeO_2. The reaction was followed by TLC. The solution was filtered and the solvent evaporated under reduced pressured. The crude material obtained was chromatographed (hexane–Et_2O), yielding 9 mg of unreacted starting material **(4),** 23 mg of 1α-hydroxy-1-epiprobotrydial (**5,** 16%), 64 mg of 11-hydroxy-1-epiprobotrydial (**6,**

44%), and 10 mg of 11-hydroxy-1α-hydroxy-1-epiprobotrydial (**7**, 6%). 1α-Hydroxy-1-epiprobotrydial (**5**): mp 123–124°C; $[\alpha]_D^{25}$ −22.3° ($CHCl_3$, c = 7.05 10^{-3}); ν^{KBr} 3339, 3209, 2972, 2933, 2882, 1634, 1429, 1357, 1293, 1242, 1173, 1126, 1015, 932, 855, 787, 706. ^{1}H NMR: 0.85 (s, 3H, H-13), 0.95 (s, 3H, H-12), 1.27 (s, 3H, H-14), 1.34 (m, 1H, H-5), 1.54 (d, 1H, $J_{7\text{-}7'}$ = 6.0 Hz, H-7), 1.58 (d, 1H, $J_{7\text{-}7'}$ = 6.0 Hz, H-7′), 1.66 (m, 1H, H-9), 1.68 (m, 1H, H-10), 1.72 (m, 1H, H-4), 1.73 (d, 3H, $J_{11\text{-}3}$ = 1.5 Hz, H-11), 1.82 (m, 1H, H-10′), 1.86 (m, 1H, H-4′), 1.90 (m, 1H, H-15), 1.96 (m, 1H, H-15′), 5.44 (qd, $J_{3\text{-}11}$ = 1.5 Hz, $J_{3\text{-}1}$ = 5.8 Hz, 1H, H-3). ^{13}C NMR: 18.18 (q, C-11), 22.50 (q, C-12), 26.52 (t, C-4), 28.76 (q, C-13), 32.71 (q, C-14), 38.20 (t, C-15), 41.03 (s, *C-6), 42.44 (t, C-10), 46.41 (s, *C-8), 53.91 (d, C-5), 59.95 (t, C-7), 66.10 (d, C-9), 85.63 (s, C-1), 123.88 (d, C-3), 139.17 (s, C-2); (* assignments may be interchanged). Mass spectrum: *m/z* 220 (14, M^+), 202 (3, M^+-H_2O), 191 (5), 173 (1), 159 (3), 149 (7), 135 (17), 121 (22), 109 (100), 108 (36). 11-Hydroxy-1-epiprobotrydial (**6**): colorless oil, $[\alpha]_D^{25}$ −53.8° ($CHCl_3$, c = 6.5 10^{-3}); ν^{film} 3307, 2952, 1635, 1446, 1363, 1297, 1256, 991, 879, 796, 689. ^{1}H NMR: 0.84 (s, 3H, H-13), 0.99 (s, 3H, H-12), 1.09 (s, 3H, H-14), 1.35 (ddd, 1H, $J_{5\text{-}9}$ = 12.2 Hz, $J_{5\text{-}4}$ = 8.1 Hz, $J_{5\text{-}4'}$ = 4.4 Hz, H-5), 1.50 (br s, 2H, H-7 and H-7′), 1.51 (m, 3H, H-15, H-15′, and H-10), 1.70 (m, 2H, H-9 and H-4), 1.93 (ddd, 1H, $J_{4\text{-}4'}$ = 16.6 Hz, $J_{4'\text{-}5}$ = 4.4 Hz, $J_{4'\text{-}3}$ = 5.6 Hz, H-4′), 2.02 (m, 1H, H-10′), 2.58 (br dd, 1H, $J_{1\text{-}10'}$ = 15.6 Hz, $J_{1\text{-}9}$ = 7.6 Hz, H-1), 4.15 (br s, 2H, H-11 and H-11′), 5.73 (br d, $J_{3\text{-}4'}$ = 5.6 Hz, H-3). ^{13}C NMR: 22.22 (q, C-14), 25.74 (t, C-4), 28.61 (q, C-13), 30.19 (q, C-12), 31.02 (t, C-10), 40.40 (s, *C-6), 40.50 (d, C-1), 43.15 (t, C-7), 45.72 (s, *C-8), 48.68 (d, C-5), 57.50 (d, C-9), 58.85 (t, C-15), 66.84 (t, C-11), 124.34 (d, C-3), 142.26 (s, C-2); (* assignments may be interchanged). Mass spectrum: *m/z* 220 (28, M^+), 205 (32), 202 (22, M^+-H_2O), 187 (32), 159 (38), 133 (55), 105 (77), 91 (84). 11-Hydroxy-1α-hydroxy-1-epiprobotrydial (**7**); mp 51°C, ν^{KBr} 3344, 2951, 1851, 1443, 1362, 1034. ^{1}H NMR: 0.81 (s, 3H, H-13), 0.90 (s, 3H, H-12), 1.22 (s, 3H, H-14), 1.27 (ddd, $J_{5\text{-}4\alpha}$ = 12.1 Hz, $J_{5\text{-}9}$ = 12.7 Hz, $J_{5\text{-}4\beta}$ = 4.3 Hz, H-5), 1.49 (d, 1H, $J_{7\text{-}7'}$ = 13.1 Hz, H-7), 1.54 (d, 1H, $J_{7\text{-}7'}$ = 13.1 Hz, H-7′), 1.7 (d, 1H, $J_{9\text{-}5}$ = 12.7 Hz, H-9), 4.11 (d, 1H, $J_{11\text{-}11'}$ = 12.3 Hz, H-11), 4.26 (d, $J_{11\text{-}11'}$ = 12.3 Hz, H-11′), 5.74 (d, 1H, J = 4.7 Hz, H-3). ^{13}C NMR: 21.9 (q, C-13), 26.0 (t, C-4), 2.82 (q, C-12), 32.3 (q, C-14), 38.7 (t, C-10), 40.0 (s, C-6), 42.2 (t, C-15), 45.7 (s, C-8), 53.4 (d, C-5), 59.6 (t, C-7), 65.5 (t, C-11), 86.2 (s, C-1), 127.8 (d, C-3), 141.8 (s, C-2). Mass spectrum *m/z* 236 (5, M^+), 218 (M^+-H_2O), 207 (23), 203 (11), 189 (35), 161 (13), 147 (17), 134 (40), 125 (67), 121 (41), 119 (24), 95 (100).

Hydrogenation of 1α-Hydroxy-1-epiprobotrydial (5). Compound **5** (50 mg) was dissolved in a mixture of benzene–ethanol (3:1) and 450 mg of tris(triphenylphosphine)rhodium(I) chloride were added. The reaction mixture

was subjected to hydrogenation (1 kg/cm^2) for 80 hr. When starting material had disappeared by TLC, the mixture was filtered and the solvent removed. After purification, 42 mg (84%) of **20** was obtained.

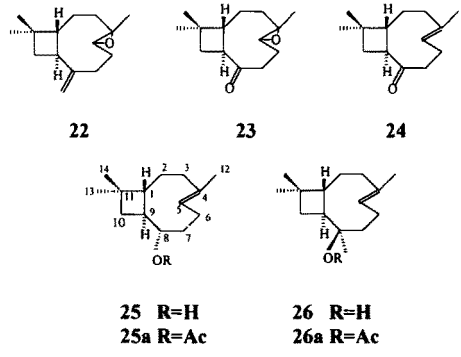
Acetylation of 11-Hydroxy-1-epiprobotrydial **(6)** *and 11-Hydroxy-1α-hydroxy-1-epiprobotrydial* **(7).** Starting materials (**6,** 30 mg; and **7,** 20 mg) were separately dissolved in dry pyridine (2 ml) and acetic anhydride (6 ml) was added. The reaction mixtures were stirred for 14 hr. The solvent was removed and the crude chromatographed to give compounds **6a** (28 mg, 78%) and **7a** (15 mg, 68%), respectively. 11-Acetyloxy-1-epiprobotrydial **(6a),** oil; ν^{film} 2952, 2868, 1735, 1364, 1223, 1019. ^{1}H NMR: 0.78 (s, 3H, H-12), 0.93 (s, 3H, H-13), 1.04 (s, 3H, H-14), 1.29 (ddd, 1H, J = 12.1, 12.4, and 3.7 Hz, H-5), 1.50 (br s, 2H, H-7 and H-7′), 1.50–1.95 (m, H-9, H-10, and H-15), 2.00 (s, 3H, CH_3CO—), 2.46 (br dd, 1H, $J_{1-10'}$ = 15.8 Hz, J_{1-9} = 7.7 Hz, H-1), 4.43 (d, 1H, $J_{11-11'}$ = 12.2 Hz, H-11), 4.48 (d, 1H, $J_{11-11'}$ = 12.2 Hz, H-11′), 5.72 (d, 1H, $J_{3-4'}$ = 5.5 Hz, H-3). ^{13}C NMR: 21.09 (c, —CO$\underline{C}$H$_3$), 22.29 (q, C-14), 25.96 (t, C-4), 28.66 (q, C-13), 30.23 (q, C-12), 30.99 (t, C-10), 40.43 (s, *C-6), 41.04 (d, C-1), 43.11 (t, C-7), 45.80 (s, *C-8), 48.41 (d, C-5), 57.36 (d, C-9), 58.84 (q, C-15), 68.19 (t, C-11), 127.82 (d, C-3), 137.35 (s, C-2), 171.01 (s, —$\underline{C}$OCH$_3$); (* assignments may be interchanged). Mass spectrum: *m/z* 262 (0.4, M$^+$), 220 (15), 202 (98), 187 (42), 173 (30), 159 (45), 146 (60), 131 (83), 118 (60), 108 (100). 11-Acetyloxy-1α-hydroxy-1-epiprobotrydial **(7a);** mp 42–43°C, ν^{KBr} 3444, 2968, 2885, 1722, 1358, 1222, 1020. ^{1}H NMR: 0.95 (s, 3H, H-12), 1.27 (s, 3H, H-14), 1.35 (dt, 1H, J = 12.2 and 4.1 Hz, H-5), 1.54 (d, 1H, $J_{7-7'}$ = 13.1 Hz, H-7), 1.59 (d, 1H, $J_{7-7'}$ = 13.1 Hz, H-7′), 1.72 (d, 1H, J_{5-9} = 12.6 Hz, H-9), 4.54 (d, J = 12.4 Hz, H-11), 4.87 (br d, $J_{11-11'}$ = 12.4 Hz, H-11′), 5.80 (br d, J = 5.3 Hz, H-3). ^{13}C NMR: 21.9 (q, $\underline{C}$H$_3$COO—), 22.5 (q, C-13), 26.7 (t, C-4), 28.7 (q, C-12), 32.7 (q, C-14), 39.3 (t, C-10), 40.9 (s, C-6=, 42.7 (t, C-15), 45.9 (q, C-8), 53.2 (d, C-5), 59.7 (t, C-7), 65.3 (t, C-11), 65.8 (d, C-9), 84.1 (s, C-1), 129.0 (d, C-3), 137.8 (s, C-2), 170.0 (s, CH$_3\underline{C}$OO—). Mass spectrum: *m/z* 278 (0.2, M$^+$), 260 (2,, M$^+$-H$_2$O), 249 (2), 218 (17, M$^+$-AcOH), 107 (100).

Acetylation of 10α-chloro-2α,3-dihydro-1α-hydroxy-1epiprobotrydial **(21).** Compound **21** (5 mg) was dissolved in acetic anhydride (0.1 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 14 hr and then extracted with AcOEt. The organic layer was neutralized with saturated $NaHCO_3$ solution, washed with brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent furnished 4 mg (78%) of the acetyl derivative, colorless oil, $[\alpha]_D^{25}$ + 19.4° ($CHCl_3$, c = 1.6 10^{-3}); ν^{film} 2926, 2861, 1728, 1452, 1367, 1233, 1126, 1029, 955, 885, 839, 719, 664. ^{1}H NMR: 0.79 (s, 3H, H-12), 0.94 (s, 3H, H-13), 1.05 (d, 3H, J_{11-2} = 7.2 Hz, H-11), 0.95–1.15 (m, 3H, H-5, H-4 and H-3), 1.15 (s, 3H, H-14), 1.45–1.55 (m, 3H, H-4, H-7, and H-7′), 1.87 (m, 1H, H-3′), 2.01 (dd, 1H, J_{10-15} = 9.4 Hz, $J_{15-15'}$ =

14.0 Hz, H-15), 2.07 (s, 3H, CH_3—CO—), 2.36 (dd, 1H, $J_{10-15'}$ = 8.8 Hz, $J_{15-15'}$ = 14.0 Hz, H-15'), 2.93 (d, 1H, J_{5-9} = 12.8 Hz, H-9), 2.98 (m, 1H, H-2), 4.59 (dd, 1H, J_{10-15} = 9.4 Hz, $J_{10-15'}$ = 8.8 Hz, H-10). ^{13}C NMR: 16.67 (q, C-11), 22.26 (q, C-13), 23.35 (q, CH_3—CO—), 24.72 (t, C-4), 28.85 (q, C-12), 31.89 (q, C-14), 34.41 (d, C-9), 35.11 (t, C-3), 40.67 (s, *C-6), 43.73 (s, *C-8), 53.64 (t, C-15), 56.95 (d, C-5), 58.05 (t, C-7), 59.86 (2C d, C-2; d, C-10), 93.02 (s, C-1), 169.49 (s, CO-CH_3) (* assignments may be interchanged). Mass spectrum *m/z* 263 (2, M^+-Cl), 240 (8, M^+-AcOH), 238 (24, M^+-AcOH), 225 (2), 223 (1), 203 (18), 182 (11), 147 (30), 131 (15), 91 (11), 69 (12), 67 (13), 55 (16), 43 (30), 40 (100).

Rearrangement of 2α,3-Dihydro-1α-hydroxy-1-epiprobotrydial ***(20)*** *with* $FSO_3H/ClFSO_2$. Compound **20** (100 mg) was subjected to the procedure described above for **18** yielding 50 mg of 1-epiprobotrydial **(4),** 10 mg of 10α-chloro-2α,3-dihydro-1α-hydroxy-1-epiprobotrydial **(21),** and 22 mg of unreacted starting material.

Alcohol ***(25)*** (Figure 3). When kobusone **(23)** was treated with Zn in HCl as described (Kaiser et al., 1986) in addition to the *nor*-caryophyllene ketone **24,** alcohol **25** (16%) was obtained. This compound was identified as its acetyl derivative **25a;** oil, ν^{film} 2924, 1857, 1735, 1446, 1368, 1230. ^{1}H NMR: 0.91 (s, 6H, H-13 and H-14), 1.35 (br t, 1H, *J* = 10.26 Hz, H-2β), 1.52 (m, 1H, H-7β), 1.62 (m, 1H, H-2α), 1.62 (m, 2H, H-12), 1.75 (m, 1H, H-6), 1.86 (br dd, 1H, *J* = 9.65 and 116.98 Hz, H-1), 1.95 (m, 1H, H-7α), 2.04 (m, 1H, H-9), 2.10 (s, 3H, CH_3CO—), 2.25 (m, 1H, H-6), 4.70 (br d, 1H, *J* = 8.33 Hz, H-8), 5.39 (br t, 1H, *J* = 7.5 Hz, H-5). ^{13}C NMR: 15.87 (q, C-12), 20.98 (q, CH_3CO—), 22.77 (t, C-6), 22.82 (q, *C-13), 28.71 (t, C-3), 29.99 (q, *C-14), 30.70 (t, C-2), 33.18 (s, C-11), 37.96 (t, C-10), 39.49 (t, C-7), 45.20 (d, C-1), 45.82 (d, C-9), 75.77 (d, C-8), 123.00 (d, C-5), 134.96 (s, C-4),



25 R=H **26 R=H**
25a R=Ac **26a R=Ac**

FIG. 3.

170.93 (s, $CH_3\underline{C}O-$) (* assignments may be interchanged). Mass spectrum: *m/z* 250 (0.17, M^+), 207 (2, M^+-Ac), 190 (13, M^+-AcOH), 175 (21), 147 (36), 134 (100), 119 (68).

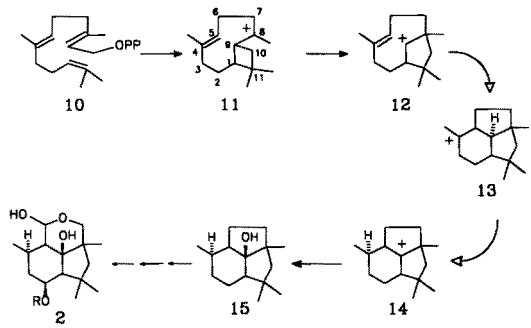
RESULTS AND DISCUSSION

Biosynthetic studies carried out by Hanson (1981) suggest that botrydial **(1)** and dihydrobotrydial **(2)** are sesquiterpenes formed from farnesyl pyrophosphate by the folding showed in Scheme 1. The first stages in the biosynthesis involve the formation and cyclization of caryophyllene cation **(11).**

On the other hand, the tricyclic olefin **4** is an epimeric derivative at C-1 of the proposed intermediate carbocation **13** (Scheme 1), which we have named 1-epiprobotrydial **(4),** and it was used as starting material for our study. Compound **4** has previously been obtained only from isocaryophyllene **(3)** (Gollnick and Schade, 1970) and an improved method has recently been reported by us (Collado et al., 1994).

Compound **4** was subjected to the chemical transformation outlined in Fig. 2 in order to obtain analogues of the intermediates **13, 14,** and **15** (Scheme 1).

Treatment of **4** with MCPBA gave oxiranes derivatives **16** and **17,** which were separated by chromatography. The structures of compounds **16** and **17** were determined by NMR spectroscopy; ^1H-^1H and ^1H-^{13}C COSY spectra allowed assignment of all the protons in both compounds. The orientation of the oxirane rings was easily assigned from the chemical shift of protons H-5 and H-1. So, in the ^1H NMR of **16,** the signals at 2.49 (m, 1H, H-1) and 1.18 (ddd, 1H, J = 12.8, 12.8, 4.8 Hz, H-5) were consistent with the α orientation of the epoxy group, while the signals at 2.25 (ddd, 1H, J = 6.8, 8.2, 9.2 Hz,



SCHEME 1.

H-1) and 1.38 (m, 1H, H-5) in compound **17** indicated a β orientation of the oxirane ring. The stereochemistry of **16** was confirmed by NOE experiments. Irradiation of the signal at 1.18 (H-5) led to the enhancement of those at 3.08 (H-3), 0.94 (H-12), while irradiation at 2.49 (H-1) enhanced the signal at 1.70 (H-9).

From **16** and **17,** the oxirane ring opening with $LiAlH_4$ afforded stereoselectively the alcohols **18** and **19,** respectively. Both had a molecular ion at *m/z* 222 and ^{13}C NMR consistent with a molecular formula of $C_{15}H_{26}O$. From the IR absorptions at 3373 and 3336 cm^{-1}, ions peaks at *m/z* 204 (M^+-18) in the MS and a quaternary carbon signal at 72.66 and 73.32 in the ^{13}C NMR spectra of **18** and **19,** respectively, the structures of epimeric alcohols were inferred. The stereochemistry of the hydroxyl group was assigned on the basis of the chemical shift observed for the proton H-9 and H-5 in both compounds. The signal corresponding to H-9 in α-alcohol **18** was clearly deshielded (+0.25) with respect to the same signal in **19,** while that the signal of H-5 in β-alcohol **19** was deshielded (+0.15 ppm) with respect to that in alcohol **18.**

In the proposed biosynthetic route (Scheme 1), the rearrangement led to a hydrogen atom appearing at C-2, thus generating the secondary methyl group in **14.** It has been reported that a 1,3-hydrogen shift occurs during the cyclization (Hanson, 1981).

Alcohols **18** and **19** were treated with $FSO_3H/ClFSO_2$ (−120°C) separately to study the hydrogen shift on the epimeric alcohols **18** and **19** and to obtain analogs of intermediates **14** and **15** (Scheme 1). When the reactions were quenched with acetone/H_2O, compounds **20** and **21** were obtained in both reactions.

The structure of **20** was confirmed by synthesis. 1-Epiprobotrydial **(4)** was subjected to oxidation with SeO_2 yielding compounds **5, 6,** and **7.** Hydrogenation of **5** with (triphenylphosphine)rhodium(I) chloride gave selectively a compound whose spectroscopic data were identical to compound **20.** On the other hand, the ^1H NMR spectra of compounds **6** and **7** showed clearly that an oxidation at C-11 and at C-11, C-1 had taken place, respectively. Hence, the signal corresponding to the methyl group on C-2, in both compounds, had disappeared. Instead, a typical signal of $-CH_2-OH$ group [4.15 (brd) **(6)** and 4.11 (d), 4.26 (d) **(7)**] was observed. Furthermore, the signal of the proton at C-1 was absent in the ^1H NMR of **7.** As expected, the signals assigned to H-11 were shifted downfield in the monoacetates **6a** and **7a,** respectively.

Compound **21** showed a molecular ion at *m/z* 256:258 and signals in its ^{13}C NMR corresponding to four methyl, four methylene, four methine, and three quaternary carbons, which were consistent with a saturated tricyclic sesquiterpene possessing a tertiary hydroxyl group and a chlorine atom with a molecular formula of $C_{15}H_{25}OCl$. When treated with acetic anhydride, compound **21** formed a monoacetate that lacked hydroxyl absorption in the IR. From

the study of ^{1}H and ^{13}C NMR spectra, structure **21** was proposed. It was supported by homo- and heteronuclear 2-D correlation experiments. The stereochemistry of chlorine and hydroxyl groups was assigned on the basis of the NOE experiment. Irradiation of the signal at 1.31 (H-14) led to enhancement of the signals at 2.01 (H-15) and 1.82 (H-9), indicating that the signal at 2.01 corresponded to the H-15 proton, which was α-oriented. Irradiation at 4.59 (H-10) enhanced the signals of protons H-11, β H-15, and H-5. These results indicated that the H-10 proton must be β-oriented confirming the structure proposed to compound **21.**

Obtaining **21,** from alcohols **18** and **19,** by treatment with superacid could be rationalized by dehydration of alcohol **20,** followed by syn addition of species Cl^{+} and OH^{-} from the reaction mixture.

In order to confirm the formation of halohydrin **21** from alcohol **20,** this compound was treated with superacid. When **20** was subjected to treatment with $FSO_3H/ClFSO_2$, compounds **21** and **4** were obtained. Furthermore, when **20** was treated with sulfuric acid, only the olefin **4** was formed. These results confirm the formation of **21** from **20** and show that the 1,2-hydrogen shift is a reversible process in this skeleton.

On the other hand, we undertook some chemical transformation on caryophyllene oxide **(22)** (Fig. 3) in order to obtain analogs compounds of the intermediate caryophyllene cation **(11)** (Scheme 1). Hence, the ozonolysis of **22** yielded kobusone **(23)** (Kaiser and Lamparsky, 1976), which was treated with Zn in HCl to give the *nor*-caryophyllene ketone **24** and the alcohol **25.** The 8-β-hydroxy caryophyllene derivative **(26)** was obtained according to the procedure described previously (Kaiser and Lamparsky, 1976).

The antifungal properties of the synthesized analogs were determined against the growth of *B. cinerea* using the poisoned food technique (Patil et al., 1986). The commercial fungicide Euparen was used as a standard for comparison in this test. Several levels of inhibition were observed. The α-alcohol **18** exhibited the maximum percent of growth inhibition followed by alcohol **6.** Compound **18** (Figure 4) showed a total inhibition for four days and reduced the growth of the fungus (68%) after seven days. The analogs with a caryophyllene skeleton displayed a different degree of activity. Compounds **22–24** had no effect on the mycelial growth. Meanwhile alcohols **25** and **26** showed complete inhibition for three days, followed by a slow growth of fungus over seven days. These results showed that the fungus was able to overcome the inhibitory effect of these alcohols. On the other hand, the tricycle olefin **4** and its derivatives **5, 16, 17, 20, 21** were inactive.

The alcohol **6** was active (Figure 5), showing prominent zones of inhibition (35–40 mm) when it was applied at 90, 150, 250, and 300 ppm, with an apparent change of fungus morphology. However its derivatives **6a, 7,** and **7a** were inactive or showed quite weak activity.

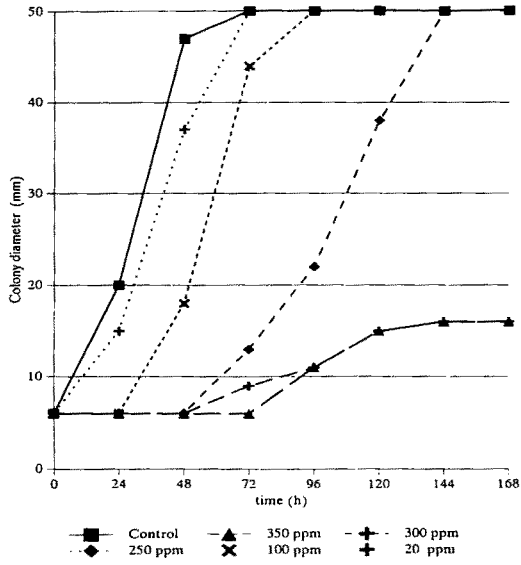


FIG. 4. Antifungal activity of alcohol **18.**

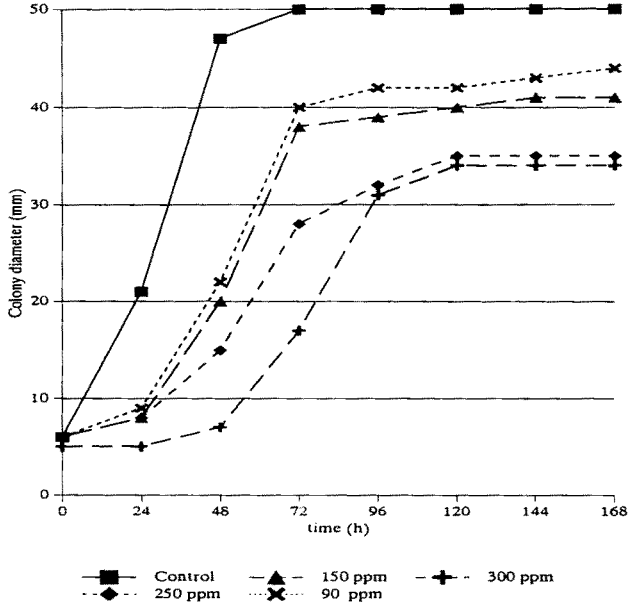


FIG. 5. Antifungal activity of compound **6.**

The study of the activity displayed by the 11-hydroxy analogs (**6** and **7**) and derivatives showed that the presence of a hydroxyl on C-1 is critical for antifungal activity of these molecules. On the other hand, it is worth noting that the acetyl derivatives of all active alcohols tested were inactive, which seems to indicate that the hydroxyl groups play an important role in the inhibition mechanism.

In conclusion, the active synthesized compounds showed fungistatic activity. The results described in this paper show that the fungus *B. cinerea* could be controlled by analogs of botrydial precursors. Work is in progress to study the mode of action of these compounds in the fungus metabolism.

Acknowledgments—This research was supported by grants from CICYT (AGR91-1021) (PB92-1101). A.J.M.S. acknowledges a fellowship from M.E.C.

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