ANTIMICROBIAL ACTIVITY OF METABOLITES FROM MYCELIAL CULTURES OF CHILEAN BASIDIOMYCETES

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ABSTRACT

We reported seven compounds isolated from mycelial cultures of Chilean Basidiomycetes. The compound structures were elucidated by spectroscopic methods. Two polyacetylenes were isolated from Gymnopilus spectabilis: hepta-4,6-diyn-3-ol (1) and 7-chloro-hepta-4,6-diyn-3-ol (2). We isolated the aromatic compounds p-anisaldehyde (3) and 3-chloro-p-anisaldehyde (4) from Hypholoma capnoides. H. fasciculare produced the compounds (3), (4), 3,5-dichloro-4-methoxy-benzyl alcohol (5) and the sesquiterpene naemotalon (6). Finally, H. sublateritium produced (5) and another sesquiterpen, marasmal (7). The compounds (1), (2), (5), and (7) showed biological activity against the tested model microorganism.

Keywords: biological activity, basidiomycetes, Chilean fungi

INTRODUCTION

Among the sources of bioactive metabolites, less intensively investigated organisms like the higher fungi seem highly promising in terms of new structures with interesting biological activities. In recent decades, interesting compounds of different biogenetic origins have been isolated from Basidiomycetes and were found to have antibacterial, antifungal, phytotoxic, nematicidal, cytostatic, antiviral, and other pharmacological activities. Basidiomycetes inhabit most climate zones, from arctic to tropical rain forest. Fruiting bodies can be collected from substrates like leaves, wood, dung, or soil.

Mycelial cultures are usually derived from spores or tissues plugs, which are allowed to germinate and grow on a solid medium consisting of yeast extract, malt extract, and glucose. Our search for novel biologically active metabolites from Chilean basidiomycetes led to previous reports of 4 new bioactive compounds, the Himanimides, from Serpula himantioides (1) and a new antifungal and cytotoxic triterpenoid, Favolon-B, isolated from fermentations of Mycena sp. (2). In the
present study, we report the results obtained from submerged cultures of Gymnopilus spectabilis, Hypholoma capnoides, H. fasciculare, and H. sublateritium.

**EXPERIMENTAL**

**Producing organisms and fermentation**

Fruiting bodies of *G. spectabilis, H. capnoides, H. fasciculare,* and *H. sublateritium* were collected in a forest close to Concepción (36° 47' SL, 73° 7' WL), Chile. Mycelial cultures were produced from spore prints of fruiting bodies. These were grown in YM medium composed of 0.4% yeast extract, 1% malt extract, 0.4% glucose, and 1.5% agar with a pH of 5.5.

Fermentations were carried out in YM medium at 24°C in a 20 liter fermentor (Braun Biostat U) with aeration (4.0 liter/air/minute) and agitation (120 rpm); 200 ml of a well-grown culture (10 days approx.) were used as inoculum. At the end of the carbon source, the fermentation was stopped and the liquid culture was separated from the mycelium by filtration. (*G. spectabilis* = 6 days; *H. capnoides* = 14 days; *H. fasciculare* = 12 days and *H. sublateritium* = 10 days).

**Isolation**

The compounds were extracted from the culture filtrate by absorption onto Mitsubishi DIAION HP-21 resin. The resin was washed with methanol and acetone, yielding crude extracts. These extracts were evaporated at reduced pressure and applied onto a column containing silica gel (Merck 60, 0.063-0.2 um; column 3 x 30 cm). Elution was carried out with hexane, ethyl-acetate, and methanol. Final purification was achieved by preparative TLC (Merck, Silica gel 60 F$_{254}$) and preparative HPLC (Jasco model PU-980 with a diode array detector; column: Macherey and Nagel, 250 x 21.2 mm containing Nucleosil C18 (7 mm) flow rate: 5 ml/min). Active compounds were isolated by bioassay-guided fractionation.

**Biological assays**

Antimicrobial activity was determined in the serial dilution assays or the plate diffusion assays as described by (3). The extracts of crude and pure substances to be tested were dissolved in the most effective solvent (100 µl/ml). Each solution (10 µl) was pipetted onto a sterile antibiotic filter disc and placed onto appropriate growth medium for the respective test organism. The crude extracts and pure compounds were tested against bacteria such as *Bacillus brevis*, *B. subtilis*, *Streptococcus pyogenes*, and *Staphylococcus aureus* and the following fungi: *Mucor miehei*, *Nematospora coryli*, *Paecilomyces variotii* and *Penicillium notatum*.

**Spectroscopic Data**

To elucidate the structures, conventional methods such as Mass, UV, IR, $^1$H-RMN, and $^{13}$C-RMN spectra were used. Mass spectra were registered with a Hewlett Packard 5890 Series II, the UV spectra were obtained with a Perkin Elmer 116, and the IR spectra with a Bruker IFS 48. $^1$H NMR were recorded at 250 MHz and $^{13}$C NMR at 65 MHz on Bruker spectrometers; chemical shifts (ppm) are related to (CH$_3$)$_4$Si as internal references.

**hepta-4,6-diyne-3-ol** (1): $^1$H NMR (CDCl$_3$) $\delta$ (ppm): 4.32(t, $^1$H), 3.45(bs, $^1$H), 2.19(s, $^1$H), 1.76-1.65(m,2H), 0.97(t,3H). $^{13}$C-RMN(CDCl$_3$) $\delta$ (ppm): 76.89(C-5), 68.67(C-7), 68.18(C-6), 67.24(C-4), 63.48(C-3), 30.22(C-2), 9.1(C-1). MS:
m/z (int. rel) 107(3), 90(23), 86(16), 79(100), 77(23), 74(18), 68(15), 62(18), 51(29).

**7-chloro-hepta-4,6-diyn-3-ol (2):** $^1$H NMR (CDCl$_3$) δ (ppm): 4.32 (t, $^1$H), 3.45 (bs, $^1$H), 2.19 (s, $^1$H), 1.76-1.65 (m, 2H), 0.97 (t, 3H). $^{13}$C NMR (CDCl$_3$) δ (ppm): 102 (C-7), 90 (C-6), 91 (C-5), 53 (C-3), 25 (C-2), 17 (C-1). MS: m/z (int. rel) 143(1), 142(1), 143(5), 125(1), 116(2), 115(32), 114(5), 113(100), 107(24), 96(5), 85(34), 77(24), 61(18), 50(29).

**p-anisaldehyde (3):** IR: $\nu_{\text{max}}$ 3077, 2969, 1698, 1601, 1578, 1511, 1461, 1427, 1394, 1302, 1261, 1216, 1151, 1109, 855, 894, 767, 645. $^1$H NMR (CDCl$_3$) δ (ppm): 9.86 (s, $^1$H), 7.82 (d, 1.7 Hz, 2H), 6.98 (d, 1.9 Hz, 2H), 3.86 (s, 3H). $^{13}$C NMR (CDCl$_3$) δ (ppm): 190.70 (s), 164.63 (s), 131.93 (d), 129.97 (s), 114.33 (d), 55.53 (q). MS: m/z (int. rel) 138(0.3), 137(2.5), 136(66), 135(69), 107(15), 92(14), 77(23), 65(9), 63(14), 51(10).

**3-chloro-p-anisaldehyde (4):** $^1$H NMR (CDCl$_3$) δ (ppm): 3.96 (s, 3H), 7.02 (d, J = 8.5 Hz, $^1$H), 7.75 (dd, J = 1.9, 8.5 Hz, $^1$H), 7.87 (d, J = 1.9 Hz, $^1$H), 9.82 (s, $^1$H). $^{13}$C NMR (CDCl$_3$) δ (ppm): 56.26 (q), 111.44 (d), 123.43 (s), 130.00 (s), 130.29 (d), 130.93 (d), 159.54 (s), 189.42 (d). MS: m/z (int. rel) 169(100), 154(1), 141(12), 126(18), 111(11), 99(20), 77(21), 63(31), 50(10).

**3,5 dichloro-4 methoxybenzyl alcohol (5):** $^1$H NMR (CDCl$_3$) δ (ppm): 4.5 (s, 3H), 7.5 (s, 2H), 2.7 (s, 2H). $^{13}$C NMR (CDCl$_3$) δ (ppm): 43.5 (t), 78.9 (q), 128 (d), 130 (d), 152 (s), 156 (s), 157.2 (s). MS: m/z (int. rel) 206 (100), 171 (94), 141 (56), 128 (25), 111 (25), 108 (30), 101 (20), 99 (73), 77 (37).

**Naematolon (6):** $^1$H-NMR (CDCl$_3$) δ (ppm): 2.24 (dd, J = 11.3, 10 Hz, $^1$H), 4.76 (dd, J = 11.3, 5.7 Hz, $^1$H), 6.26 (dq, J = 9, 1.5 Hz, $^1$H), 5.83 (dd, J = 9, 0.5 Hz, $^1$H), 3.24 (dddd, J = 10, 10, 5.4, 0.8 Hz, $^1$H), 1.16 (s, 3H), 1.36 (s, 3H), 1.79 (dd, J = 1.4, 0.5 Hz, $^1$H), 5.77 (d, J = 0.8 Hz, $^1$H), 5.83 (br s, $^1$H), 2.16 (s), 3.64 (d, J = 5.7 Hz, $^1$H).

**Marasmal (7):** IR: $\nu_{\text{max}}$: 3465, 3264, 1754, 1687, 655, 1632, 1370, 1365. $^1$H NMR (CDCl$_3$) δ (ppm): 9.36 (s, CHO), 7.15 (br d, H-7), 5.31 (br s, $^1$H), 4.34 (br dd, J = 2.8, 2.8 Hz, $^1$H), 3.69 (m, $^1$H), 3.51 (br dd, J = 2.8, 2.8 Hz, $^1$H), 2.43 (br dd, J = 19.4, 7.0, 3.5, 1.5 Hz, $^1$H), 2.12 (br dd, J = 12.8, 3.0, 2.0 Hz, $^1$H), 1.95 (dd, J = 14.8, 2.8, 2.8 Hz, $^1$H), 1.87 (dd, J = 12.8, 3.5 Hz, $^1$H), 1.13 (s, 3H), 0.99 (s, 3H). $^{13}$C NMR (CDCl$_3$) δ (ppm): 195.2 (d), 178.9 (s), 156.2 (d), 140.4 (s), 101.5 (d), 77.9 (d), 70.8 (d), 54.6 (s), 47.7 (d), 38.2 (s), 37.3 (d), 30.4 (q), 28.9 (t), 25.8 (q), 23.0 (t). MS: m/z (int. rel) 260 (M$^+$ - H$_2$O, 15), 250 (20), 234 (9), 232 (35), 206 (23), 188 (25), 157 (38), 151 (25), 143 (36), 135 (46), 133 (37), 119 (27), 117 (33), 107 (27), 69 (44), 67 (20), 65 (22), 55 (29), 53 (27).

**RESULTS AND DISCUSSION**

All reported compounds are known and were isolated from the filtrated liquid (see Figure 1). Two polyacetylenes were isolated from *G. spectabilis*: hepta-4,6-diyn-3-ol (1) and 7-chloro-hepta-4,6-diyn-3-ol (2). These compounds are probably derived by stepwise desaturation of saturated fatty acids using crepenyic acid as key intermediaries in the biosynthesis of many polyacetylenes in fungi (4). The strong biological activity of compounds (1) and (2) can be traced back to their reactive
triple bonds (5). The antimicrobial activities of the pure compounds are presented in Table 1.

**Figure 1.** Structures of compounds isolated from some Chilean Basidiomycetes.

_H. capnoides_ produced _p_-anisaldehyde (3) and 3-chloro-_p_-anisaldehyde (4); _H. fasciculare_ (3), (4) 3,5-dichloro-4-metoxi-benzyl alcohol (5) and naematolon (6) and _H. sublateritium_ (5) and marasmal (7).
The known compounds (3), (4) and (5) have been isolated from mycelial cultures and from natural substrates of several common-wood and forest-litter degrading fungi, e.g. *Pleurotus pulmonaris*, *Bjerkandera adusta*, and *Pholiota squarrosa* (6,7). These compounds showed negative activity against the bacteria and fungi tested. Compounds (6) and (7) are fungal sesquiterpenes formed via the humulane-protoilludane biosynthesis pathway. The caryophyllane derivative naematolon (6) has also been isolated from fermentations of several *Hypholoma* (*Naematoloma*) species and *Panus* strains (8). Naematolon showed weak antibacterial and antifungal activity. On the other hand, marasmal, possessing the marasmane skeleton, has previously been reported from liquid cultures of the fungi *Marasmius oreades* (9). This compound was preferentially active toward the fungi tested as compared to bacteria.

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