NEW BUTYROLACTONE FROM A MARINE-DERIVED FUNGUS ASPERGILLUS SP

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ABSTRACT

Four compounds that belong to two structure types, namely dibenzylbutyrolactone and sesterterpenoids, were obtained from the extract of the strain Aspergillus sp. (2P-22), isolated from a marine sponge, Cliona chilensis. Among them, compound 1 was identified as new, namely butyrolactone-VI. The structures of these compounds were characterized on the basis of spectroscopic data. Biological activities of these fungal metabolites, are described.

Keywords: marine-derived fungus, Aspergillus sp. (2P-22); dibenzylbutyrolactone; sesterterpenoids.

INTRODUCTION

In the search for novel and bioactive molecules, terrestrial fungi have yielded many biologically active compounds. More recently, marine microorganisms have gained attention as important sources of chemically interesting and biologically active secondary metabolites, due to the diversity in chemical structures and biological activities1-2. However, compared with other marine organisms, relatively few investigations of the secondary metabolites from marine fungi have been reported3. Fungi isolated from various organisms in the marine environment, e.g., from mangroves4, algae5, mollusks6 and particularly sponges7, have been examined for their secondary metabolite content.

As part of our research on marine fungi, we report here the results regarding the secondary metabolite chemistry of 2P-22 strain, Aspergillus sp., isolated from the marine sponge Cliona chilensis collected in Los Molles, IV Region, Chile. This study led to the isolation of a new compound 1, together with three known compounds previously isolated from other fungal sources 8-11 (Figure 1). The new compound is structurally related to the known butyrolactone I isolated from Aspergillus terreus12. We described the isolation, structure elucidation, and biological activities of these fungal metabolites.

EXPERIMENTAL

General experimental procedures.

1H and 13C-NMR spectra were recorded on a Bruker AMX2-400 spectrometer, operating at 400.13 for 1H and 100.6 MHz for 13C. Chemical shifts are reported in ppm (δ) and coupling constant (J) are given in Hz. The spectra were obtained in CDCl3, solutions and are referred to the residual peaks of CHCl3, at δ 7.26 ppm and 77.0 ppm for 1H and 13C respectively. Mass spectra were taken at 70 eV (probe) in a Micromass Autospec spectrometer. Semipreparative HPLC was carried out with a Beckman System Gold 125P. Dry column chromatography was performed on Merck (0.02-0.063 mm) silica gel.

Fungal Material.

The fungal isolate was recovered from the surface of the sponge Cliona chilensis collected in the Pacific Sea, Los Molles (IV Región, Chile), during September 2007. A sample of this strain was deposited in the Laboratory of Marine Natural Products of the Universidad de Chile coded as 2P-22 and kept in agar slants with potato dextrose agar (PDA) as culture medium. The strain was seeded in Petri dishes with PDA culture medium in seawater and incubated for 7 days at 28°C. Then, a solution of Tween 80 (0.05%) in sterile distilled water was used to obtain a spore suspension. This suspension was poured into an Erlenmeyer flask containing 150 mL of Czapek medium (5g of yeast extract, 0.5g of MgSO4, 0.5g of KH2PO4, 1L of seawater) and cultured at 27°C with 230 rpm for 48 h to prepared a stock inoculum. The large-scale fermentation was performed in Erlenmeyer flask (10 x 1L each) containing 200 mL of Czapek medium and 20 mL of inoculum. The cultures were incubated on an orbital shaker (230 rpm) at 27°C for 25 days.

The mycelium was removed from the culture broth by filtration. Both the broth and the mycelium were studied. First, the broth (10 L) was extracted with EtOAc, (3 x 1.5 L). The resulting organic extract was dried under reduced pressure to obtain a brown solid (0.5 g), which was fractionated by column chromatography on silica gel using stepwise gradient elution from 70% hexane in ethyl acetate, to 100% ethyl acetate, to 100% methanol. The volume eluted in each step was 0.5 L, and 10 fractions were obtained and evaporated to dryness. Moreover, the dried mycelium (12 g) was first extracted with CHCl3, (3 x 1 L) to give 1 g of dichloromethane extract and then with CH3OH (3 x 1 L) to obtain 11 g of methanol extract. Purification of the resulting fractions of the organic culture broth allowed us to obtain the pure compounds 1-4 as follows: the methanol extract was fractionated on a Sephadex LH-20 column using a 6:2:1 n-hexane/CH3Cl/MeOH solvent system to obtain 20 fractions. Fraction 10 (100 mg) was further separated using silica gel (200-300 mesh) CC with...
a gradient solvent system from 50% petroleum ether/EtOAc to 100% EtOAc to yield 27 fractions (50 mL each for fraction). The fractions were monitored by TLC. Further purifications were made by chromatography on silica gel eluting with fractions 1 and 2. From the purification by chromatography on silica gel using mobile phase hexane/EtOAc (8:3), fractions 15 and 18 contained compounds 3 and 4 respectively. Compounds 3 - 4 were identified by comparison of their spectroscopic properties with those reported \(^{39,41}\).

**Compound (1):** 'H-NMR (400 MHz); 7.59 (2H, d, J = 8.8 Hz, H-5' and H-9'), 6.90 (2H, d, J = 8.8 Hz, H-6' and H-8'), 6.58 (1H, brs, H-5), 6.55 (1H, d, J = 8.3 Hz, H-8), 6.52 (1H, dd, J = 2.0, 8.3 Hz, H-9), 4.94 (1H, t, J = 4.9 Hz, H-11), 3.78 (3H, s, OCH3), 3.76 (1H, t, J = 4.9 Hz, H-11), 3.53 (1H, dd, J = 14.7 Hz, H-3a), 3.45 (1H, d, J = 14.7 Hz, H-3e), 2.88 (1H, dd, J = 4.6, 17.0 Hz, H-10a), 2.62 (1H, dd, J = 5.1, 17.0 Hz, H-10c), 1.31 (3H, s, Me-13c), 1.24 (3H, s, Me 14). \(^{13}C-NMR:** 169.7 (C-1), 85.9 (C-2), 38.5 (C-3), 128.0 (C-4), 131.2 (C-5), 118.2 (C-6), 151.9 (C-7), 116.7 (C-8), 129.6 (C-9, 31.0) (C-10), 69.6 (C-11), 72.2 (C-12), 24.6 (C-13), 14.9 (C-14), 127.4 (C-15), 124.7 (C-13), 128.0 (C-14), 129.5 (C-5', C-9') and 116.0 (C-6' and C-8'), 156.6 (C-7').

**RESULTS AND DISCUSSION**

Chromatographic separation of compounds extracted from the culture of 2P-22 strain, Aspergillus sp. led to the isolation of 4 compounds 1-4: one new, 1, and three known compounds, named butyrolactone 1, \(^{21}\), and the two sesterterpenoids tereteronin A, 3 and tereteronin B, \(^{44}\). The structures of the known compounds were confirmed by comparing their spectroscopic properties with those reported \(^{28,41}\).

**Compound 1** was obtained as colourless crystals. Its molecular formula was established as \(C_{16}H_{23}O_7\). The \(^{1}C\) NMR and DEPT spectra of 1 showed proton signals for two methyl, one methoxy, two methylene, eight methines and 11 quaternary carbon atoms including two carbonyl carbons and a sp\(^2\) carbon bearing a hydroxyl group. The \(^1\)H-NMR spectra of 1 displayed signals for a 1,4-disubstituted phenolic moiety at 8.759 (2H, d, J = 8.8 Hz) and 6.90 (2H, d, J = 8.8 Hz), three aromatic proton signals at 7.26, 7.02 (2H, d, J = 8.8 Hz and 6.8 Hz), 6.62 (1H, dd, J = 1.5, 8.5 Hz), 6.58 (1H, d, J = 8.5 Hz, H-5'), 6.48 (1H, brs, H-5), 4.94 (1H, t, J = 5.2 Hz, H-11), 3.78 (3H, OCH3), 3.76 (1H, t, J = 4.6 Hz, H-11), 3.45 (1H, dd, J = 14.7 Hz, H-3a), 3.42 (1H, d, J = 14.7 Hz, H-3e), 2.88 (1H, dd, J = 4.6, 17.0 Hz, H-10a), 2.60 (1H, dd, J = 5.1, 17.0 Hz, H-10c), 1.31 (3H, s, Me-13c), 1.24 (3H, s, Me 14). The comparison of the 

**Functional (contraction/relaxation) studies in rat isolated thoracic aorta**

Endothelium-denuded vascular rings were prepared from aortae of male Wistar rats weighing 210-270 g, essentially as described elsewhere \(^{17}\). From the purification by chromatography on silica gel eluting with fractions 1 and 2, the two sesterterpenoids tereteronin A, 3 and tereteronin B, \(^{44}\). The structures of the known compounds were confirmed by comparing their spectroscopic properties with those reported \(^{28,41}\).

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**Functional (contraction/relaxation) studies in rat isolated thoracic aorta**

Endothelium-denuded vascular rings were prepared from aortae of male Wistar rats weighing 210-270 g, essentially as described elsewhere\(^{17}\). Contraction studies were performed following the general procedure previously reported \(^{18}\). In the present study, however, contraction responses were recorded by means of force-displacement transducers (Dynamometer UF1, Pioden Controls Ltd.,) connected to a MacLab\(^{TM}\) system (ADInstruments Pty Ltd.), controlled by a Power Macintosh 5500-225 computer. After an equilibration period of at least 1 h, isotonic contractions induced by L-phenylephrine (1 \(\mu\)M) were obtained. When contraction of the tissue in response to this vasoconstrictor agent had stabilized (after about 20 min), cumulatively increasing concentrations of the tested compounds (1-200 \(\mu\)M) were added to the bath at 5-10 min intervals (the time needed to obtain steady-state relaxation). Control tissues were subjected to the same procedures simultaneously, but omitting the compounds and adding the vehicle (appropriate dilutions of DMSO).

Contraction responses to phenylephrine are expressed as a percentage of the maximal contraction produced by this vasoconstrictor agent before the addition of the tested compounds. Sigmodial concentration-response curves for the vasorelaxant effects of the tested compounds were fitted using the program Origin\(^{TM}\) 7.0 (Microcal Software, Inc., Northampton, USA), with estimation of IC\(_50\) values (i.e. concentrations inducing 50% relaxation) for phenylephrine-induced contractions.

Significant differences between two means (p < 0.05 or p < 0.01) were determined by one-way analysis of variance (ANOVA) followed by the Dunnett's post-hoc test.

Appropriate dilutions of the above drugs were prepared every day immediately before use in deionized water from the following concentrated stock solutions kept at -20°C: the tested compounds (100 mM) in DMSO, L-phenylephrine (100 mM) in deionized water. In our experiments, neither deionized water nor appropriate dilutions of the vehicle used had significant pharmacological effects.
BIOLOGICAL ACTIVITY

The result of the antibacterial activity assay shows that 1 and 2 were only active against Clavibacter michiganensis. The MIQ value of butyrolactone VI 1 was 50 µg. The crown gall tumor bioassay used to test the compounds for antitumor activity was performed essentially according to McLaughlin & Rogers15. It has been demonstrated that the compounds which inhibit these plant tumors have a high predictability of showing activity against the P288 (3PS) leukaemia in mice16 and, in general, of disrupting the cell cycle (mitosis, G phase, etc.) regardless of their mode of action17. Compounds 1 and 2 showed a similar and significant inhibition of the growth of crown gall tumors on potato discs. The values were: butyrolactone I 1 (84, 46%) and butyrolactone VI 2 (71, 64%), which suggests in vivo antitumor activity for both compounds.

Butyrolactone 1 2 was acetylated as usual (Py/acetic anhydride) yielding compound 2a. The potential vasorelaxant effects of compounds 2 and 2a were evaluated on endothelium-denuded rat aortic rings precontracted with L-phenylephrine.4,11 L-phenylephrine caused slow and sustained contraction of the rat isolated aortic rings without endothelium. The maximal tension (mg) reached was 2053.9 ± 89 (n = 5). These contractile effects were maintained without significant tension changes in control rings for at least 90 min. DMSO had no significant effects on phenylephrine-induced contractions in endothelium-denuded rat aortic rings (n = 5, p > 0.05). The cumulative addition of the tested compounds (20-200 µM) concentration-dependently relaxed the contractions induced by L-phenylephrine (Figure 2). The butyrolactone 1 acetate was slightly less efficient than butyrolactone I in relaxing these contractions. However, the corresponding IC50 values of both compounds did not present significant differences (Table 1).

CONCLUSIONS

In summary, a new cytotoxic compound, butyrolactone VI, 1, has been isolated from a strain of Aspergillus sp., isolated from a Chilean sponge Cliona chilensis. Its structure resembles that of other butyrolactones, previously characterized from marine strain of Aspergillus terreus and Aspergillus sp.18-11. These compounds were tested for antibacterial and antitumor assays and for potential vasorelaxant effects obtaining good responses suggesting that further studies to assess the potential of this type of structure are merited.

REFERENCES


Figure 2. (a). Cumulative concentration-relaxation curves for the tested compounds (20-200 µM) in endothelium-denuded rat thoracic aortic rings pre-contracted with phenylephrine (1 µM). (b) Each point represents the mean value ± s.e.m (indicated by vertical lines) from 5 experiments. (c) Level of statistical significance: *p < 0.01 o **p < 0.05 with respect to the maximal tension (100%), as determined by ANOVA/Dunnett’s.

Table 1. IC50 values (µM) for the vasorelaxation induced by the tested compounds in endothelium-denuded rat aortic rings pre-contracted with L-phenylephrine.

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<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
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<tr>
<td>2</td>
<td>91.6 ± 7.1 (n=6)</td>
</tr>
<tr>
<td>2a</td>
<td>121.7 ± 9.8 (n=5)</td>
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Each value represents the mean ± s.e.m from experiments shown in brackets.