Palmaenones A and B, Two New Antimicrobial Chlorinated Cyclopentenones from Discomycete Lachnum palmae

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Two new antimicrobial chlorinated cyclopentenones, palmaenones A (1) and B (2) were isolated from the culture broth of discomycete Lachnum palmae (NBRC-106495), and the structures of 1 and 2 were elucidated by spectroscopic data and the stereochemistry of 1 was directly determined by a single-crystal X-ray diffraction analysis. Palmaenones A (1) and B (2) are cyclopentenones containing three chlorines. Compound 1 exhibited potent antimicrobial activity against Micrococcus luteus, Mycobacterium smegmatis, Escherichia coli, Xanthomonas campestris, and Macrococcus racemosus, while the activities of compound 2 were weaker than 1.

Key words Lachnum palmae; chlorinated cyclopentenone; palmaenone A; palmaenone B; Discomycete; antimicrobial activity

For the purpose of screening of new bioactive metabolites, fungi of the order Leotiales, Discomycetes, which have been underutilized for microbial screenings, were collected and isolated. Therefore, we noticed discomycete fungi as a source for new bioactive substances. In our screening for antimicrobial activity of various strains of discomycete fungi in the National Museum of Nature and Science, Lachnum palmae was selected. A series of the genus Lachnum is one of the most popular and remarkable inperculate discomycetes. The genus of Lachnum is known to embrace some 150 species and yet more members have been added to science. The antimicrobial and nematicidal pentaketide compounds have been isolated from Lachnum papyraceum. In our recent research two new chlorinated dibenzo-alpha-pyrones, palmiariols A and B, have been isolated from the mycelial extracts of L. palmae. In this paper, we describe the isolation and structure elucidation of two new chlorinated cyclopentenones, palmaenones A (1) and B (2), from culture broth of L. palmae and their antimicrobial activities.

The discomycete L. palmae was grown in PYG broth [polypeptone (1%), yeast extract (0.5%), and glucose (2%) in water, pH 7.5] at 23 °C for 1 week. The culture broth was centrifuged and the supernatant was extracted with EtOAc. The EtOAc extract was subjected to silica gel column chromatography and reversed-phase HPLC to yield two new chlorinated cyclopentenones, palmaenones A (1) and B (2).

Palmaenone A (1) showed pseudomolecular ion peaks at m/z 297 [M–H]–, 299 [M+2–H]–, 301 [M+4–H]–, and 303 [M+6–H]– (27 : 27 : 9 : 1) in the electrospray ionization (ESI)-MS, indicating the presence of three chlorine atoms in 1. The molecular formula of 1 was deduced as C10H9Cl3O4 from high resolution (HR)-ESI-MS [m/z 296.9483 (M–H)–, Δ 0.5 mmu]. The IR spectrum indicated the presence of hydroxyl (3410 cm−1), ester (1749, 1213 cm−1), and unsaturated carbonyl (1637 cm−1) groups, while the UV absorptions at 242 and 290 nm implied that 1 possessed an α,β-unsaturated ketone. The gross structure of 1 was deduced from detailed analysis of the 1H- and 13C-NMR data (Table 1) aided by 2D-NMR experiments (1H–1H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC)) (Fig. 2). The 13C-NMR data indicated that the molecule possessed one unsaturated carbonyl carbon, one ester carbonyl carbon, four conjugated olefin carbons, one methine carbon, one oxygenated quaternary carbon, one methoxy carbon, and one methyl carbon (Table 1). The UV and 13C-NMR spectra of 1 were similar to those of cryptosporiopsin, VM 4798-1a, and VM 4798-1b. The 1H–1H COSY connectivities between H-1 and H-2 as well as the HMBC correlations of H-2 (δH 6.68) to C-3 (δC 122.8) and C-4 (δC 157.5) indicated that the two carbon–carbon double bonds were conjugated. The HMBC correlation of H-7 (δH 4.64) to C-6 (δC 187.5) revealed a carbonyl group was attached at C-6. HMBC correlations of H-7 to C-4 and C-8 (δC 83.1) and OH-8 (δH 4.52) to C-4, C-7 (δC 65.3), and C-8 indicated that C-4 was connected to C-8. The HMBC correlations of a methoxy proton (δH 3.85) to C-7 (δC 157.0) revealed that the carboxymethoxy group was connected to C-8. HMBC correlations of H-1 and H-2 to C-3...
and OH-8 to C-7, and comparison of the $^{13}$C-NMR data of 1 with that of cryptosporiopsin$^9$ revealed the presence of three chlorine atoms at C-3, C-5, and C-7. Compound 1 was crystallized in monoclinic space group $P2_1/c$ (#14) and the $Z$-geometry of the trisubstituted double bond at C-2—C-3 and trans geometry between OH-8 and Cl-7 were deduced from the X-ray data (Fig. 3). However, compound 1 was racemic compound, as indicated by the symmetry operations of space group $P2_1/c$ (#14), in which each asymmetric unit comprises two molecules of 1 of the same chirality with slight structural differences (Fig. 4). Thus the structure of palmaenone A was assigned as 1.

Palmaenone B (2) showed pseudomolecular ion peaks at $m/z$ 297 [M—H]$^-$, 299 [M+2—H]$^-$, 301 [M+4—H]$^-$, and 303 [M+6—H]$^-$ (27:27:9:1) in the ESI-MS. The molecular formula of 2 was deduced as C$_{10}$H$_9$Cl$_3$O$_4$ from HR-ESI-MS [$m/z$ 296.9480 (M—H)$^-$, D$_2$O 0.8 mmu], indicating that 2 was an isomer of 1. The IR spectrum indicated the presence of hydroxyl (3450 cm$^{-1}$), ester (1751, 1214 cm$^{-1}$), and unsaturated carbonyl (1639 cm$^{-1}$) groups, while the UV absorption at 242 and 287 nm implied that 2 possessed an $\alpha,\beta$-unsaturated ketone. The gross structure of 2 was deduced by detailed analysis of the $^1$H- and $^{13}$C-NMR data (Table 1) aided by 2D-NMR experiments ($^1$H–$^1$H COSY, HMQC, and HMBC) (Fig. 5). The UV, $^1$H-, and $^{13}$C-NMR spectra of 2 were similar to those of palmaenone A (1) except for signals of H-2 ($\delta_H$ 6.32, 1H, q, $J$=7.6 Hz), C-2 ($\delta_C$ 135.5), and C-3 ($\delta_C$ 119.0) and the appearance of nuclear Overhauser effect spectroscopy (NOESY) correlations of H-2 and H-1 ($\delta_H$ 1.75) to MeO-9 ($\delta_H$ 3.86) (Fig. 5). These data suggested that palmaenone B (2) is a stereoisomer of palmaenone A (1) and the E-geometry of trisubstituted double bond at C-2—C-3. Compound 2 may be also racemic by considering of the same biogenesis as 1. Thus the structure of palmaenone B was assigned as 2.

Palmaenones A (1) and B (2) are cyclopentanones with three chlorines at C-3, C-5, and C-7, and should be pentaketides in polyketide pathway. $^9$ Although compounds 1 and 2 are racemic, the antipode compounds, (+)-cryptosporiopsin and (+)-mycorrhizin A, of (−)-cryptosporiopsin and (−)-mycorrhizin A, which are related analogues of 1 and 2, have been reported.$^7$ In 1969, natural dichlorinated cyclopentenones, cryptosporiopsin and related compounds were iso-

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### Table 1. $^{13}$C- and $^1$H-NMR Data of Palmaenones A (1) and B (2) in CDCl$_3$

<table>
<thead>
<tr>
<th></th>
<th>$\delta_C$</th>
<th>$\delta_H$, mult. ($J$ in Hz)</th>
<th>HMBC$^{(a)}$</th>
<th></th>
<th>$\delta_C$</th>
<th>$\delta_H$, mult. ($J$ in Hz)</th>
<th>HMBC$^{(a)}$</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td>Palmaenone B (2)</td>
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<td>1</td>
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<td>2, 3</td>
<td>1</td>
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<td></td>
<td>4, 7, 8, 9</td>
</tr>
</tbody>
</table>

$^a$ HMBC correlations are from proton(s) stated to the indicated carbon.

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![Fig. 3. ORTEP Drawing of Palmaenone A (1)](image)

![Fig. 4. Two Molecules of Palmaenone A (1) with the Antipode of 1](image)

![Fig. 5. 2D-NMR Correlations of Palmaenone B (2)](image)
labeled from Cryptosporiopsis sp.,10 Sporormia affinis,9 and Periconia macrosporina11) and the antibiotic activity of cryptosporiosis against plant pathogenic fungi and other microorganisms was reported.12) Palmaenones A (1) and B (2) are the first examples of natural trichlorinated cyclopentenones.

Palmaenones A (1) and B (2) were evaluated for antimicrobial activity against four Gram-positive bacteria, five Gram-negative bacteria, four fungi, and one yeast by plate diffusion assay (Table 2). Compound 1 showed strong antimicrobial activity against Micrococcus luteus, Mycobacterium smegmatis, Escherichia coli, Xanthomonas campestris, and Mucor racemosus, while the activities of compound 2 were weaker than 1.

Experimental

General Procedures Optical rotations were recorded on a Jasco DIP-370. UV spectra were recorded on a HITACHI U-2000A spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. 1H- and 13C-NMR spectra were measured and recorded on a Bruker Avance 500 spectrometer. 1H and 13C-NMR spectra were used as internal references for the 1H- and 13C-NMR spectra. ESI-MS were recorded on a JEOL JMS-T100LC mass spectrometer.

Suitable Single-Crystal X-Ray Crystallography of Palmaenone A (1) Suitable colorless platelets of 1 were obtained from a solution of acetone. The crystal (0.16 x 0.12 x 0.02 mm3) belongs to the monoclinic system, space group P21\(/a\), with a = 11.2049(11) Å, b = 11,9961(11) Å, c = 10,537(2) Å, \( \beta = 116.1558(8)^\circ \), \( V = 1267.2(2) \) Å\(^3\), Z = 4, \( D_{calcd} = 1.570 \) g/cm\(^3\), and (CuK\( \alpha \) = 0.6580 Å). Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer to \( \theta_{max} = 136.4^\circ \). All 19790 reflections were collected. The structure was solved by direct methods (SHELX97) and refined with full-matrix least-squares on \( F^2 \) using anisotropic thermal parameters. Hydrogen atoms were located by difference Fourier techniques and refined with isotropic thermal parameters. The refined structural model converged to a final \( R = 0.0401; wR = 0.1078 \) for 2288 observed reflections \( [I > 2.00 \sigma(I)] \) and 158 variable parameters. Crystallographic data of 1 have been deposited with the Cambridge Crystallographic Data Centre (deposit no. CCDC 838643). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2, 1EZ, U.K. (fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

Antimicrobial Assay Antibiotic activity against four Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, and Mycobacterium smegmatis), five Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Xanthomonas campestris, Bacteroides fragilis, and Acholeplasma laidlawii), and four fungi (Pycnuria oryzae, Aspergillus niger, Mucor racemosus, and Candida albicans) and one yeast (Saccharomyces cerevisiae) were tested by plate diffusion assay using 6 mm paper disk. Palmaenones A (1) and B (2) solutions (1 mM) were prepared by dissolving each compound in acetone. Each adjusted solution was added in paper disk (5 or 20 μl) and paper disk were drying. The paper disks were set on the agar plate suspended tested microorganisms. After cultivating microorganisms at 37°C for 72 h, the strength of antimicrobial activity was estimated by measuring the diameter length of inhibition zone (mm).

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References