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Phomactin H, a novel diterpene from an unidentified marine-derived fungus

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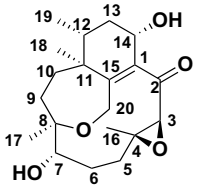
Abstract—Phomactin H (**1**), a diterpene has been isolated from cultures of an unidentified marine-derived fungus. The structure and relative stereochemistry of phomactin H (**1**) was determined by X-ray diffraction analysis. Phomactin H (**1**), having an oxepane moiety, is a new phomactin with a novel skeleton.

Marine-derived fungi have become a rich source of structurally novel and pharmacologically active secondary metabolites. In the natural product literature, the distribution of fungi from a marine algae is only about 10% of the total number of marine-derived fungi, but algicolous fungi are second, accounting for 27% of the total number of new compounds reported from marine-derived fungi (sponge-derived fungi; 28%).[1] Therefore, algicolous fungi represent a valuable resource in the search for structurally novel compounds. In this letter, we describe the isolation and structure elucidation of a novel phomactine H (**1**).



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An unidentified fungus (MPUC 046) was isolated from the surface of the marine brown alga *Ishige okamurae*, collected at Tateishi, Kanagawa Prefecture, Japan, in September 2000. The D1/D2 26S rDNA and internal transcribed spacer regions including 5.8S rDNA in the rRNA gene of the isolate were directly sequenced using a PCR product. The sequence data (approximately 1200 bp long) were searched using the BLAST system (http://www.ncbi.nlm.nih.gov/BLAST/) at GenBank. As the isolate was not assigned to any known species, it belongs to Dothideales, phylogenetically. MPUC046 was not closely related to *Phoma* sp. producing phomactins on a molecular phylogenetic tree.

Fermentation was carried out in ten 500-mL Roux flasks each containing 150 g wheat. Seawater (40 mL) was added to each flask and the contents were soaked for 40 min before autoclaving for 30 min. The flasks were inoculated with the strain (MPUC 046) and incubated at 25 °C for 31 days. The fermented wheat substrate was extracted with $CHCl_3$ (2×2.8 L) and the combined extracts were filtered and evaporated to yield 20.98 g of crude extract. The $CHCl_3$ extract (12.0 g) was subjected to silica gel column chromatography and then HPLC to afford **1** (21.6 mg).

Phomactin H (**1**), colorless prism; $[\alpha]_D^{20}$; +204.0 (*c* 0.18, MeOH); UV λ_{max} (MeOH) nm ($\log \varepsilon$): 250 (3.84), was suggested to have the molecular formula $C_{20}H_{30}O_5$ by its HREIMS data *m/z* 350.2087 $[M]^+$, (calcd 350.2093). The IR spectrum exhibited the absorptions due to

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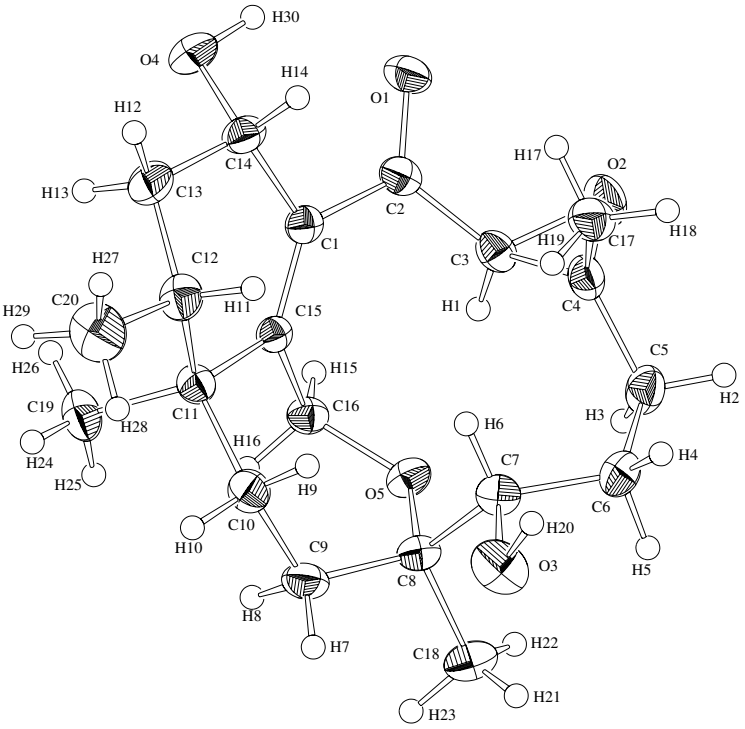

Figure 1. X-Ray crystal structure of phomactin H (**1**).

hydroxyl (3520 cm^{-1}) and carbonyl (1640 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** displayed line broadening at room temperature or at 60 °C and the ^{13}C NMR spectrum displayed no signal of more than 50 ppm. Therefore, the structure of **1** was difficult to determine by ^{1}H and ^{13}C NMR spectra. Fortunately, colorless crystals of **1** could be obtained for X-ray crystallographic analysis, which confirmed the structure of **1** (Fig. 1).[2]

Thus far only nine phomactins are known (A,[3] B, B1, B2, C, D,[4] E, F, and G[5]). All phomactins have been isolated from *Phoma* sp. As mentioned above, the fungus MPUC046 was not assigned to any known species and it belongs to Dothideales, phylogenetically. The fungus MPUC046 was not closely related to *Phoma* sp. on a molecular phylogenetic tree. Therefore, a morphological classification of the fungus MPUC046 is interesting. Since compound **1**, containing the oxepane skeleton was a new phomactin with novel skeleton, compound **1** was named phomactin H. Phomactins are reported to be active as PAF (platelet activating factor) antagonists. Additional structure–activity relationships were investigated after synthetic modifications to produce a larger series of phomactin congeners.[6] Phomactin H (**1**) with novel skeleton is interesting in whether a PAF antagonist action is shown like other phomactins.

Acknowledgements

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References and notes

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2. X-ray crystal structure analysis of **1**: crystal data: colorless prismatic crystal of $C_{20}H_{30}O_5$. Space group = $P2_12_12_1$, $a = 13.031(1)$, $b = 13.510(2)$, $c = 10.316$ (2) Å, $V = 1816.2(4)$ Å^3, $Z = 4$, crystal size: $0.50 \times 0.44 \times 0.30$ mm. A total of 1898 unique reflections ($2\theta < 135.88°$) were collected using graphite monochromated CuKα ($\lambda = 1.54178$ Å) on a Rigaku AFC7S diffractometer. The structure was solved by direct methods (SIR-97) and expanded using Fourier techniques (DIRDIF94). The final cycle of full-matrix least squares refinement was based on 1898 unique reflections ($2\theta < 135.88°$) and 226 variable parameters and converged with unweighted and weighted agreement factors of $R = 0.095$, $R_w = 0.118$ and $R_1 = 0.039$ for $I > 2.0\sigma(I)$ data. Crystallographic data (excluding structure factors) for structure **3** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 238187. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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