Phomactin A: A Novel PAF Antagonist from a Marine Fungus *Phoma* sp.

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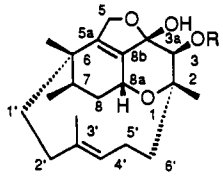
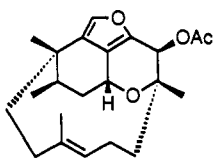
PAF (platelet activating factor, 1-*O*-alkyl-2(*R*)-(acetylglyceryl)-3-phosphorylcholine) causes platelet aggregation, chemotaxis, and degranulation of polymorphonuclear leukocytes, smooth muscle contraction, vascular permeability, and hypotension.[1] Recent studies have shown that PAF may be involved in many inflammatory, respiratory, and cardiovascular diseases. Recently we reported a specific PAF antagonist, chatancin,[2] isolated from a soft coral *Sarcophyton* sp., and a variety of synthetic[1] and natural[3] antagonists has been found. In our program for finding PAF antagonists from marine sources, we focused on fungal isolates from the marine environment, since little is known of their metabolites.[4] We systematically screened lipophilic extracts of marine fungal isolates for inhibition of PAF-induced platelet aggregation and binding of PAF to its receptors and found that a marine fungus *Phoma* sp.[5] produced a novel PAF antagonist, phomactin A. Here we report the isolation, characterization, and PAF antagonistic activities of phomactin A (**1a**).



1a R=H
1b R=Ac
1c R=COC$_6$H$_4$Br



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Phoma sp. (SANK 11486) was isolated from the shell of a crab,[6] *Chionoecetes opilio*, collected off the coast of Fukui prefecture, Japan. Culture filtrate (12 L)[7] of this fungus, cultivated at 26 °C for 7 days, was extracted with ethyl acetate (12 L). Assay-directed purification of the EtOAc extract by silica gel and reverse-phase chromatography gave phomactin A (3.0 mg).

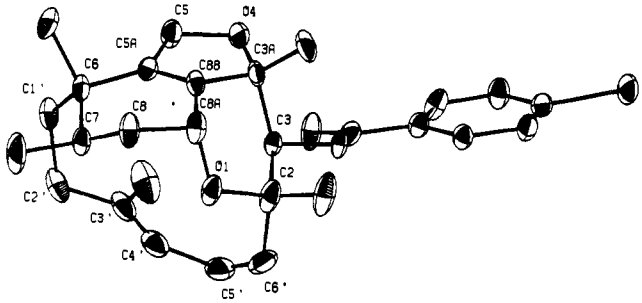
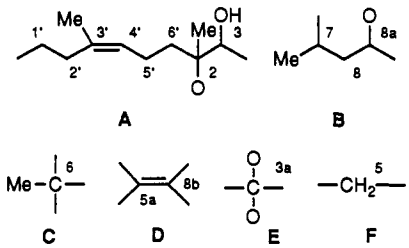
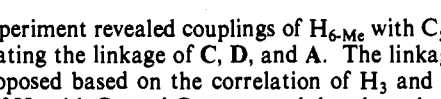


Figure 1. ORTEP drawing of **1c** at the 30% probability level.

Phomactin A (**1a**)[8] has the molecular formula $C_{20}H_{30}O_4$ based on high-resolution mass spectral data (HREIMS, *m/z* 334.2125; Δ −1.8 mmu). The IR spectrum showed the presence of a hydroxy group (3400 cm^{-1}), and the UV spectrum had no absorption maximum. The ^{1}H and ^{13}C NMR spectra[8] indicated the presence of two double bonds, one ketal, a secondary methyl, two tertiary methyls, and an olefinic methyl. A secondary alcohol was confirmed by a down field ^{1}H NMR shift upon acetylation to give **1b** (3.57 to 5.00 ppm). A ^{1}H-^{1}H COSY experiment revealed the presence of the partial structures **A**-**F**. A long-range ^{1}H-^{13}C



A **B**



C **D** **E** **F**

COSY experiment revealed couplings of $H_{6\text{-}Me}$ with C_{5a} and $C_{1'}$, demonstrating the linkage of **C**, **D**, and **A**. The linkage of **A** to **E** was proposed based on the correlation of H_3 and C_{3a}. The coupling of H_5 with C_{5a} and C_{3a} suggested that these three carbons and C_{8b} constituted a dihydrofuran ring. This was confirmed by formation of compound **2**[9] upon dehydration of **1a** with acetic anhydride and (dimethylamino)pyridine (DMAP). The correlation of **B** and **C** was unclear, because the coupling of $H_{6\text{-}Me}$ and C_7 was not observed, while that of H_7 and C_6 was observed. Since

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(5) *Phoma* sp. may be the anamorph of *Trichomaris invadens*: Hibbits, J.; Hughes, G. C.; Sparks, A. K. *Can. J. Bot.* **1981**, *59*, 2121-2128.

(6) Sporulation occurred only on marine agar medium (not observed without sea water), though growth and metabolite production do not always require sea water.

(7) Medium for production of phomactin A: sucrose 2%, K_2HPO_4 0.5%, peeled and mashed potato 10%, peptone 1% in artifical sea water (Jamarin-S), pH 8.5.

(8) **1a**: oil, $[\alpha]_D$ + 175° (*c* 0.75, $CHCl_3$), ν_{max} ($CHCl_3$) 3400, 1580, 1450, 1380, 1230, 1050, 960, 760 cm^{-1}; ^{1}H NMR (500.2 MHz CD_3OD) 5.37 (1 H, br d, *J* = 11.9 Hz, 4'-H), 4.63 (1 H, dd, *J* = 12.8, 1.5 Hz, 5-H), 4.47 (1 H, d, *J* = 12.8 Hz, 5-H), 4.06 (1 H, td, *J* = 2.7, 1.5 Hz, 8a-H), 3.57 (1 H, s, 3-H), 2.77 (1 H, ddq, *J* = 12.5, 4.0, 7.0 Hz, 7-H), 2.43 (1 H, ddd, *J* = 14.7, 13.6, 4.0 Hz, 2'-H), 2.41 (1 H, ddt, *J* = 15.3, 5.0, 12.5 Hz, 5'-H), 1.97 (1 H, ddd, *J* = 14.7, 4.0, 3.3 Hz, 2'-H), 1.93 (1 H, dddd, *J* = 15.3, 5.0, 2.6, 1.5 Hz, 5'-H), 1.74 (1 H, ddd, *J* = 15.4, 13.6, 3.3 Hz, 1'-H), 1.72 (1 H, ddd, *J* = 14.6, 5.0, 2.6 Hz, 6'-H), 1.67 (1 H, ddd, *J* = 14.2, 4.0, 2.7 Hz, 8-H), 1.65 (3 H, t, *J* = 1.5 Hz, 3'-CH_3), 1.62 (1 H, ddd, *J* = 14.2, 12.5, 2.7 Hz, 8-H), 1.60 (1 H, ddd, *J* = 14.6, 12.5, 5.0 Hz, 6'-H), 1.51 (1 H, dt, *J* = 15.4, 4.0 Hz, 1'-H), 1.22 (3 H, s, 2-CH_3), 0.92 (3 H, d, *J* = 7.0 Hz, 7-CH_3), 0.90 (3 H, s, 6-CH_3); ^{13}C NMR (125.8 MHz, CD_3OD) 144.6 (s, 5a-C), 131.4 (d, 4'-C), 131.3 (s, 8b-C), 128.7 (s, 3'-C), 110.0 (s, 3a-C), 81.3 (s, 2-C), 74.6 (d, 3-C), 72.0 (t, 5-C), 63.0 (d, 8a-C), 38.6 (t, 6'-C), 38.0 (s, 6-C), 37.6 (t, 2'-C), 34.5 (t, 1'-C), 34.2 (t, 8-C), 27.8 (d, 7-C), 25.8 (t, 5'-C), 21.9 (q, 6Me-C), 19.6 (q, 2Me-C), 16.5 (q, 3'Me-C), 14.9 (q, 7Me-C).

(9) **2**: $C_{22}H_{30}O_4$ (HREIMS, *m/z* 358.2137; Δ −0.7 mmu), λ_{max} (EtOH) 225 nm (ε 6000), ν_{max} ($CHCl_3$) 2950, 1740, 1460, 1370, 1220, 1040, 1020, 960 cm^{-1}; ^{1}H NMR (270.2 MHz, $CDCl_3$) 7.05 (1 H, s), 5.88 (1 H, s), 5.02 (1 H, br s), 4.92 (1 H, dd, *J* = 9.5, 7.8 Hz), 2.28-2.43 (2 H, m), 2.08 (3 H, s), 1.70-2.21 (9 H, m), 1.37 (3 H, s), 1.28 (3 H, s), 1.19 (3 H, s), 1.00 (3 H, d, *J* = 7.3 Hz); ^{13}C NMR (67.9 MHz, $CDCl_3$) 170.7 (s), 143.4 (s), 138.9 (d), 138.0 (s), 130.5 (s), 127.0 (s), 125.1 (d), 80.5 (s), 68.7 (d), 64.1 (d), 42.9 (t), 41.5 (s), 39.0 (d), 35.9 (t), 35.7 (t), 35.1 (t), 28.0 (q), 26.7 (8), 23.4 (t), 20.9 (q), 17.7 (q), 16.6 (q).

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these data were not enough to determine the structure of **1a**, X-ray analysis was performed on a mono-*p*-bromobenzoyl derivative (**1c**).[10] The structure was determined by the direct method (MULTAN 78) and successive block-diagonal least-squares and Fourier synthesis. Parameters were refined by using anisotropic temperature factors to $R = 0.052$ for 1985 reflections [$|F_o| > 3\sigma(F_o)$]. Nineteen Bijvoet pairs which exhibited large effects of anomalous scattering from the bromine atoms were selected and used to determine the absolute configuration. All observed Bijvoet rations were in agreement with the ones calculated for the chosen enantiomer in Figure 1. Consequently, phomactin A is (2*S*,3*S*,3a*S*,6*S*,7*R*,8a*R*)-3,3a-dihydroxy-2,6-(3′-methyl-3′-hexeno)-2,6,7-trimethyl-3a,5,6,7,8,8a-hexahydrofuro[2,3,4-*de*]chroman (**1a**). Some similar metabolites possessing a [9.3.1]pentadecane ring have been isolated from higher plants,[11] but this macrocyclic furochroman ring has not been found in natural products.

Phomactin A inhibited PAF-induced platelet aggregation (IC_{50} 1.0×10^{-5} M) and binding of PAF to its receptors (IC_{50} 2.3×10^{-6} M) but had no effect on adenosine diphosphate, arachidonic acid, and collagen-induced platelet aggregation. Thus, phomactin A is a new type of specific PAF antagonist. It is interesting that phomactin A has a glycerin-like subunit at C_2, C_3, and C_{3a}, since PAF itself has a glyceryl unit. This part may account for the activity. *Phoma* sp. produced many other phomactin derivatives, some of which were 10–100 times as active as **1a**. Structural analysis, derivatization, and structure-activity studies of these compounds are underway. Details will be reported elsewhere.

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Supplementary Material Available: Experimental procedures and X-ray analysis data (16 pages). Ordering information is given on any current masthead page.

(10) **1c**: space group $P2_12_12_1$, $a = 9.239$ (1) Å, $b = 16.894$ (3) Å, $c = 15.9000$ (2) Å, $V = 2481.8$ (5) Å, $Z = 4$, $D_C = 1.38$ g/cm³, μ(Cu Kα) = 27.8 cm⁻¹.

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Bifunctional Monomolecular Langmuir–Blodgett Films at Electrodes. Electrochemistry at Single Molecule "Gate Sites"

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Control of reactivity at the electrode/solution interface through control of chemical and structural features of interfacial films has been a major goal in electrochemistry.[1] Below, we describe a monomolecular surface assembly of long chain amphiphilic molecules which allows us to channel access to the electrode surface through a controlled number of single molecule "gate sites". The monolayer assembly is bifunctional in its structure and properties and consists of two types of molecules, those that passivate the electrode and those which open access to its surface. Octadecanethiol ($C_{18}SH$) and octadecyl hydroxide ($C_{18}OH$) are used together to block access to the electrode.[2] Ubiquinone (Q_{10}), a long-chain benzoquinone derivative shown below, acts as a gate site. The monolayer film is initially assembled at the air/water interface where it is compressed and then deposited at the electrode surface by the Langmuir–Blodgett (L-B) method.[2]

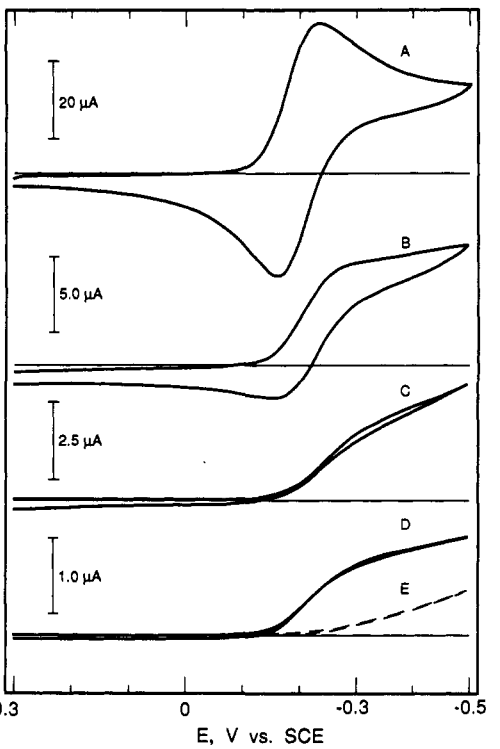
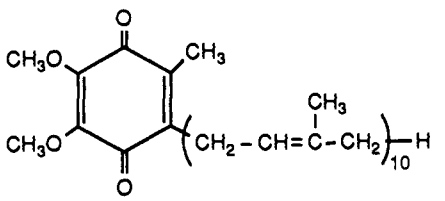


Figure 1. Cyclic voltammograms of $Ru(NH_3)_6^{3+}$ (1.0 mM in 0.5 M KCl) at a bare gold electrode (A) and those coated with $Q_{10}/C_{18}SH/C_{18}OH$ L-B monolayers ($x_{C_{18}SH}:x_{C_{18}OH} = 2.3$, L-B transfer pressure, 20 mN/m). Surface concentrations of Q_{10} (in mol/cm²): B, 2.5×10^{-15}; C, 5.4×10^{-17}; D, 1.9×10^{-17}; E, 0. $A = 0.40$ cm², $v = 50$ mV/s.



Q_{10}

Formation of monolayers of long-chain surfactants on solid surfaces can be accomplished by the L-B method or via spontaneous self-assembly.[3] Densely packed self-assembled alkyl thiol monolayers were investigated as model organic surfaces[4] and used in the studies of long-range electron-transfer kinetics,[5] where compact alkyl thiol layers provided tunneling barrier of adjustable thickness.[6] Coassembly of octadecanethiol and a nonamphiphilic component on gold electrodes led to the formation of surface assemblies with ion-selective[7] or catalytic properties.[8] In comparison with self-assembly, L-B techniques offer two important advantages. One is a broader range of applications, since one is not limited to thiol derivatives. The other is precise control of composition of mixed monolayer assemblies. We showed recently that monomolecular L-B films consisting of ca. 70 mol% of $C_{18}SH$

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