COMMUNICATIONS TO THE EDITOR

Phomactin E, F, and G: New Phomactin-group PAF Antagonists from a Marine Fungus *Phoma* sp.

Sir:

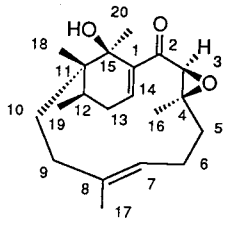
Platelet activating factor (PAF) is a naturally occurring etherphospholipid that is a mediator of anaphylaxis released by a number of stimulated cells, such as basophils, neutrophils, platelets, and macrophages, and it causes degranulation of polymorphonuclear leukocytes, smooth muscle contraction, vascular permeability, and hypotension.[1] Intensive efforts to find drugs that attenuate the effects of PAF have resulted in the discovery of a number of specific PAF antagonists, some of which are being tested for their clinical effectiveness.[2,3]

In the course of finding PAF antagonists from metabolites of a marine fungus *Phoma* sp., we have discovered novel PAF antagonists: phomactin A, B. B_1, B_2, D.[4,5] CHU *et al.* also reported several PAF antagonists with the same skeletal framework as phomactins.[6,7] Recently we found three related compounds; phomactin E (**1**), F (**2**), and G (**3**). In this paper, we report the structures and PAF antagonistic activities of **1**, **2**, and **3**.

Fermentation was carried out at 23°C with agitation at 80 rpm for 12 days in two 600-liter tanks, each containing 300 liters of a medium consisting of sucrose 2.0%, K_2HPO_4 0.5%, peptone 1.0%, potato 1.0%, and CB442 0.02% (pH 8.5).

Culture filtrate (600 liters) was extracted with EtOAc (600 liters). Assay-directed purification of the EtOAc extract by silica gel and reversed-phase chromatography gave phomactin E (**1**) (940.0 mg), F (**2**) (25.0 mg) and G (**3**) (170.0 mg). The ^1H and ^{13}C NMR (Tables 1 and 2) and other spectral data (Table 3) showed that these compounds had the same skeletal framework as phomactin B (**4**).

The molecular formula of phomactin E ($C_{20}H_{30}O_3$), determined by high-resolution mass spectrum (HREI-MS, *m/z* 318.21684; Δ −2.6 mmu), was less by one oxygen than that of **4** ($C_{20}H_{30}O_4$). Comparison of NMR spectra of **4** and **1** showed that one carbon containing a



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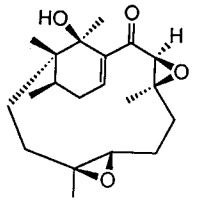

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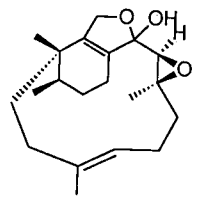

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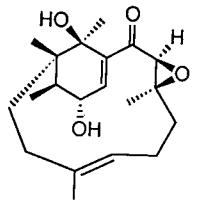

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Table 1. ^1H NMR spectra of phomactin E (**1**), F (**2**), G (**3**), and B (**4**) (CD_3OD).

Number	(1) ppm (mult., *J*, Hz)	(2) ppm (mult., *J*, Hz)	(3) ppm (mult., *J*, Hz)	(4) ppm (mult., *J*, Hz)
3	3.91 (s)	3.99 (s)	2.85 (s)	3.81 (s)
5	1.24 (m)	1.38 (m)	1.92 (m)	1.24 (m)
	2.17 (m)	2.23 (m)	1.72 (m)	2.15 (m)
6	2.46 (m)	2.21 (m)	1.89 (m)	2.38 (m)
	2.12 (m)	1.48 (m)	1.79 (m)	2.10 (m)
7	5.37 (br t, 7.2)	2.73 (dd, 10.5, 3.7)	5.07 (br d, 10.0)	5.31 (br t, 7.3)
9	2.20 (m)	1.59 (m)	2.45 (m)	2.16 (m)
	2.20 (m)	1.59 (m)	1.94 (m)	2.16 (m)
10	1.35 (m)	1.99 (m)	1.67 (m)	1.39 (ddd, 15.7, 7.6, 4.6)
	2.17 (m)	1.03 (m)	1.53 (m)	2.02 (ddd, 15.7, 8.0, 4.9)
12	1.61 (dq, 8.5, 7.5)	1.74 (dq, 9.3, 7.3)	2.13 (ddq, 12.0, 2.5, 7.3)	1.63 (dq, 3.2, 7.5)
13	2.78 (ddd, 20.6, 8.5, 3.5)	2.49 (ddd, 19.4, 9.3, 6.4)	1.64 (dq, 12.5, 12.0)	4.12 (dd, 3.2, 2.6)
	2.05 (dd, 20.6, 4.3)	2.10 (dd, 19.4, 2.7)	1.44 (m)	
14	5.86 (dd, 3.5, 4.3)	5.84 (dd, 6.4, 2.7)	2.01 (m)	5.91 (d, 2.6)
			1.85 (m)	
16	1.20 (s)	1.27 (s)	1.40 (s)	1.24 (s)
17	1.63 (s)	1.32 (s)	1.64 (s)	1.60 (s)
18	1.17 (s)	1.15 (s)	0.93 (s)	1.13 (s)
19	1.23 (d, 7.3)	1.18 (d, 7.3)	0.92 (d, 7.3)	1.27 (d, 7.5)
20	1.48 (s)	1.49 (s)	4.51 (ddq, 12.5, 2.0, 1.5)	1.46 (s)
			4.37 (ddd, 12.5, 4.1, 1.3)	

Table 2. ^{13}C NMR spectra of phomactin E (**1**), F (**2**), G (**3**), and B (**4**).

Number	1 ppm (mult.)	2 ppm (mult.)	3 ppm (mult.)	4 ppm (mult.)
1	149.5 (s)	150.6 (s)	134.5 (s)	147.2 (s)
2	203.2 (s)	202.6 (s)	109.7 (s)	200.3 (s)
3	68.8 (d)	68.2 (d)	64.9 (d)	65.9 (d)
4	64.2 (s)	64.4 (s)	61.4 (s)	62.8 (s)
5	39.2 (t)	35.5 (t)	35.1 (t)	37.4 (t)
6	24.4 (t)	25.3 (t)	24.4 (t)	22.7 (t)
7	121.0 (d)	62.5 (d)	129.5 (d)	120.3 (d)
8	137.8 (s)	64.0 (s)	135.2 (s)	136.8 (s)
9	33.9 (t)	38.9 (t)	36.1 (t)	33.6 (t)
10	35.0 (t)	35.6 (t)	33.6 (t)	36.7 (t)
11	44.0 (s)	43.7 (s)	38.6 (s)	41.5 (s)
12	40.9 (d)	34.3 (d)	34.5 (d)	46.3 (d)
13	32.9 (t)	33.4 (t)	29.1 (t)	71.4 (d)
14	134.4 (d)	133.4 (d)	24.1 (t)	135.6 (d)
15	74.2 (s)	76.5 (s)	144.7 (s)	73.3 (s)
16	15.3 (q)	14.8 (q)	21.0 (q)	14.5 (q)
17	17.9 (q)	16.2 (q)	17.0 (q)	16.4 (q)
18	19.0 (q)	21.1 (q)	22.2 (q)	19.7 (q)
19	21.1 (q)	22.0 (q)	14.9 (q)	19.7 (q)
20	26.0 (q)	23.2 (q)	70.5 (t)	23.2 (q)

1, **2**, **3** were measured in CD_3OD. **4** was measured in DMSO-d_6.

Table 3. Physicochemical properties of phomactin E (**1**), F (**2**), and G (**3**).

	1	2	3
MP (°C)	148~149	199~202	131~132
Molecular formula	$C_{20}H_{30}O_3$	$C_{20}H_{30}O_4$	$C_{20}H_{30}O_3$
HREI-MS	318.21684	334.21322	318.21765
$[\alpha]_D^{25}$ ($CHCl_3$)	+178.4	+120.9	+96.9
UV λ_{max} (ε)	238 (3500)	239 (3200)	End

hydroxyl (δ_C 71.4 (d), δ_H 4.12 (1H dd, J=3.2, 2.6 Hz)) in **4** was replaced by a methylene carbon ((δ_C 32.9 (t), δ_H 2.78 (1H, ddd, J=20.6, 8.5, 3.5 Hz), 2.05 (1H, dd, J=20.6, 4.3 Hz)). Furthermore, the long-range coupling was observed from the doublet methyl proton (δ_H 1.23) to this methylene carbon. **1** was therefore suggested to be a deoxy derivative of **4** at C_{13}. In order to verify the proposed structure and establish the overall stereochemistry of **1**, X-ray diffraction analysis was performed on a crystal obtained from CH_2Cl_2 - hexane (space group $P22_12_1$, a=20.382 (1) Å, b=13.498 (1) Å, c=13.391 (2) Å, Z=8). 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=0.060. The ORTEP of **1** is shown in Fig. 1.

The X-ray analysis revealed a further structural feature in that α,β-unsaturated ketone (C_{14}-C_1-C_2-O) was nonplanar; the dihedral angle between the carbonyl and the $\Delta^{1,14}$ double bond was 119°. This accounted for the

Fig. 1. ORTEP drawing of phomactin E (**1**).

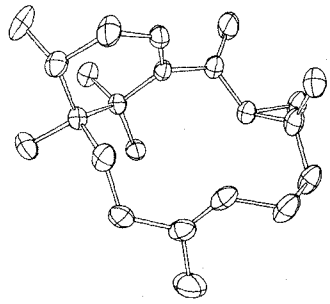
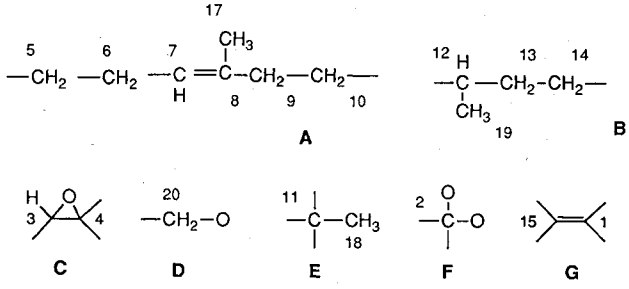


Fig. 2. Partial structures of phomactin G (**3**).



low ε (3500) in the UV, and an unusual ^{13}C NMR assignment at C_1 (δ_C 149.5) and C_{14} (δ_C 134.4).

Phomactin F (**2**) has the molecular formula $C_{20}H_{30}O_4$, determined by HREI-MS (m/z 334.21322 Δ −1.1 mmu). In the ^1H and ^{13}C NMR, **2** differed from **1** only in the replacement of one double bond (δ_C 121.0 (d), 137.8 (s); 5.37 (1H, br t, J=7.2 Hz) in **1** by a epoxide (δ_C 64.0 (s), 62.5 (d); δ_H 2.73 (1H, dd, J=10.5, 3.7 Hz) in **2**. The UV spectrum showed the maximum at 239 nm (ε 3200) due to the enone (C_{14}-C_1-C_2-O). Combining of the above data suggested that **2** had the epoxide at C_7 and C_8. To confirm the structure, **1** was converted to **2** by MCPBA oxidation.

The MCPBA oxidation of **1** in $CHCl_3$ gave a single diastereomer in an 87% yield, suggesting that the oxidation occurred at the less hindered side of the double bond. From the ORTEP drawing of **1**, the 8-*Si* plane at C_7, C_8 was more open and therefore preferred by the attack of MCPBA to give **2**. To clarify the conformation of **1** in $CHCl_3$, NOE experiments were performed. The irradiation of H_3 resulted in enhancement of H_{20}, whereas the irradiation of H_7 resulted in enhancement of H_{16}, H_{14}, and H_{13}. These results showed that **1** had the same conformation in $CHCl_3$ as in crystal state. Hence the structure of phomactin F, including the stereochemistry at C_7 and C_8, is proposed as **2**.

Phomactin G (**3**) has the molecular formula $C_{20}H_{30}O_3$, from its HREI-MS (m/z 318.21765 Δ −1.8 mmu). The ^1H, ^{13}C NMR, and DQF COSY experiments inferred the partial structure **A**~**G** (Fig. 2). Further information regarding the skeletal framework was sought from the COLOC experiment. The cross peak of $3H_{18}$ to C_{12},

C_{15}, and C_{10} confirmed the linkage **E**, **B**, **G**, and **A**. The linkage **A** and **C** was obtained from the coupling of $3H_{16}$ with C_5 and C_3. The couplings of H_{20} with C_{15}, C_1, and C_2 suggested that **D**, **G**, and **F** constituted a dihydrofuran ring. Insertion of this dihydrofuran ring between C_3 and C_{14} was based on the coupling of H_3 to C_2 and H_{14} to C_1. Based on these data, the structure of phomactin G was proposed as **3**. The stereochemistries at C_3, C_4, C_{11}, and C_{12} were assumed to be same as **1** because **1** and **3** may have the same biosynthetic pathway. However the stereochemistry at C_2 is still unknown.

Phomactin E (**1**), F (**2**), and G (**3**) inhibited PAF-induced platelet aggregation with IC_{50}s of 2.3 μM, 3.9 μM, and 3.2 μM, respectively, and also inhibited binding of PAF to its receptors with IC_{50}s of 5.19 μM, 35.9 μM, and 0.38 μM, respectively. **2** is less active than **1** due to the presence of epoxide at C_7, C_8. These data suggested that the lipophilicity at C_7, C_8 had a significant role in receptor binding.

The absolute stereochemistry of **1**, **2**, and **3** is now under investigation.

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