Phomactins, Novel PAF Antagonists from Marine Fungus *Phoma* sp.

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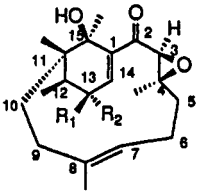
Diterpenes phomactins B (**1a**), B1 (**1c**), B2 (**2a**), C (**4**), and D (**5**) were isolated from a marine fungus, *Phoma* sp. These structures were determined based on spectroscopic evidences, X-ray crystallography, and chemical conversion. We also determined the absolute configuration of **1a**, **1c**, and **2a** by the advanced Mosher's method and circular dichroism. Phomactin D inhibited the binding of PAF to its receptors and PAF-induced platelet aggregation with IC_{50} of 1.2×10^{-7} M and 8.0×10^{-7} M, respectively, while other compounds antagonized PAF action at higher concentrations.

Marine organisms have produced a variety of chemically and physiologically intriguing compounds. Recently, however, more attention has being paid to marine microorganisms, including bacteria, cyanobacteria, fungi, and microalgae.[1] The marine fungi are generally named for fungi adapted to the marine environment. They are taxonomically diverse and quite different from terrestrial ones ecologically, morphologically, and physiologically. The marine fungi, having an obscure inherent metabolism, have frequently been difficult to isolate, and chemical study of their secondary metabolites has proceeded much slower in comparison with terrestrial microorganisms.

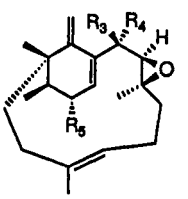
In order to reveal the secondary metabolite-producing potential of marine fungi and to isolate physiologically active compounds from them, we have systematically tested their extracts for many bioassay systems to find compounds of biological significance. We recently reported the chemical characterization of a novel PAF antagonist, phomactin A (**3**), isolation from a culture broth of marine fungus *Phoma* sp. (SANK 11486).[2] And from the extract from this fungus we obtained many other phomactin-related compounds. Herein we report the isolation, characterization, and PAF-antagonistic activities of the following novel compounds: phomactins B (**1a**), B1 (**1c**), B2 (**2a**), D (**5**),[3] together with known compound C (**4**) (Sch 47918).[4]

Phoma sp. was isolated from the shell of a crab, *Chinoecetes opilio*, collected off the coast of Fukui prefecture, Japan. The culture filtrate (600 L) of this fungus, cultivated at 23 °C for 13 days,[5] was extracted with ethyl acetate (800 L). Assay-directed purification of the EtOAc extracts on silica gel and reversed-phase chromatography gave phomactins B (2103.0 mg), B1 (46.0 mg), B2 (677.0 mg), C (1008 mg), and D (84.2 mg), respectively.

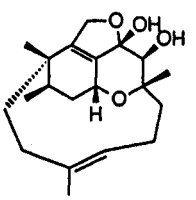
Phomactin B (**1a**) has the molecular formula $C_{20}H_{30}O_4$, from its high-resolution mass spectrum (HREIMS, *m/z*



1a; R_1=H, R_2=OH
1b; R_1=R_2=O
1c; R_1=OH, R_2=H
1d; R_1=H, R_2=O(+)MTPA
1e; R_1=H, R_2=O(-)MTPA



2a; R_3=R_4=O, R_5=OH
2b; R_3=R_4=O, R_5=OAc
2c; R_3=OH, R_4=H, R_5=OH
2d; R_3=OH, R_4=H, R_5=OBz



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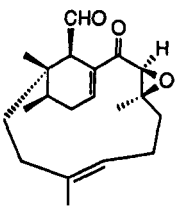

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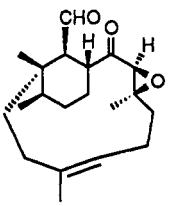

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§ Analytical and Metabolic Research Laboratories.
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(3) (a) Sugano, M.; Sato, A.; Oshima, T.; Furuya, K.; Kuwano, H.; Hata, T.; Hanzawa, H.; Yoda, K.; Haruyama, H. *Tennen Yuki Kagoubutsu Toronkai Koen Yoshishuu* **1991**, *33*, 699-706 (Japan). (b) Sugano, M.; Sato, A.; Oshima, T.; Furuya, K.; Haruyama, H. Jpn. Kokai Tokkyo Koho JP 91216197.

(4) (a) Chu, M.; Patel, M. G.; Gullo, V. P.; Trumess, I.; Puar, M. S. *J. Org. Chem.* **1992**, *57*, 5817-5818. (b) Chu, M.; Trumess, I.; Gunnarsson, I.; Bishop, W. R.; Kreutner, W.; Horan, A. C.; Patel, M. G.; Gullo, V. P.; Puar, M. S. *J. Antibiot.* *46*, 554-563.

(5) Medium for production of phomactins B, B1, B2, C, and D; sucrose 2%, K_2HPO_4 0.5%, peptone 1%, peeled and mashed potato, 10%, in artificial sea water (Jamarin-S), pH 8.5. This temperature and time are best for the production of phomactin A and B.

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Table 1. ^{1}H NMR Spectrum of Phomactin B (CD_3OD)

number	^{1}H, ppm	(mult, J, Hz)	DQF COSY	relayed COSY
3	3.81	(s)		
5a	2.15	(m)		
b	1.24	(m)	6a	7
6a	2.38	(m)	5b, 7	
b	2.10	(m)		
7	5.31	(br t, J = 7.3 Hz)	6a,8Me	5b
9a	2.16	(m)	10a, 10b	
b	2.16	(m)	10a, 10b	
10a	2.02	(ddd, J = 15.7, 8.0, 4.9 Hz)	9a, 9b	11Me
b	1.39	(ddd, J = 15.7, 7.6, 4.6 Hz)	9a, 9b	
12	1.63	(dq, J = 3.2, 7.5 Hz)	12Me, 13	14
13	4.12	(dd, J = 3.2, 2.6 Hz)	12, 14	12Me
14	5.91	(d, J = 2.6 Hz)	13	12, 15Me
4Me	1.24	(s)		
8Me	1.60	(s)	7	
11Me	1.13	(s)		10a
12Me	1.27	(d, J = 7.5 Hz)	12	13
15Me	1.46	(s)		14

Table 2. HMBC Experiment for Phomactin B (DMSO-d_6)

number	^{13}C, ppm (mult)	long range correlation to H no.
1	147.2 (s)	13, 15Me, 15-OH
2	200.3 (s)	3, 14
3	65.9 (d)	5a, 5b, 4Me
4	62.8 (s)	3, 4Me, 5a, 5b, 6a, 6b
5	37.4 (t)	3, 4Me, 6a, 6b
6	22.7 (t)	5a, 5b, 7
7	120.3 (d)	5a, 5b, 6a, 6b, 8Me, 9a, 9b
8	136.8 (s)	6a, 6b, 8Me, 9a, 9b, 10a, 10b
9	33.6 (t)	7, 8Me, 10a, 10b
10	36.7 (t)	9a, 9b, 11Me, 12
11	41.5 (s)	9a, 9b, 10a, 11Me, 12, 15Me, 15-OH
12	46.3 (d)	10a, 11Me, 13, 14
13	71.4 (d)	10a, 12
14	135.6 (d)	13
15	73.3 (s)	10a, 11Me, 12, 14, 15Me, 15-OH
4Me	14.5 (q)	
8Me	16.4 (q)	7, 9a, 9b
11Me	19.7 (q)	10a, 10b
12Me	19.7 (q)	12, 13
15Me	23.2 (q)	15-OH

334.21330; Δ −1.0 mmu). The IR spectrum showed the presence of hydroxy groups [ν_{max} (KBr) 3420, 3380 cm^{-1}], and the UV spectrum [λ_{max} (EtOH) 240 nm (ϵ 3100)] supported the presence of an enone. The ^{1}H (Table 1) and ^{13}C NMR spectra (Table 2) indicated the presence of a ketone [δ_C 200.3 (s)], two double bonds [δ_C 147.2 (s), 120.3 (d), 136.8 (s), 135.6 (d), δ_H 5.91 (1H, d, J = 2.6 Hz), 5.31 (1H, brt, J = 7.3 Hz)], and two carbons containing hydroxy groups [δ_C 73.3 (s), 71.4 (d), δ_H 4.12 (1H, t, J = 2.9 Hz), 4.53 (1H, s), 5.10 (1H, d, J = 4.4 Hz)[6]]. The presence of an epoxide was based on the signals at C_3 [δ_C 65.9 (d),$^1J_{CH}$ = 175 Hz] in the ^{13}C NMR.

DQF COSY[7] and relayed COSY[8] experiments (Table 1) inferred the partial structures of **A**–**C** (Figure 1). Further information regarding the skeletal framework was sought from multiple bond proton–carbon couplings, identified by a H-detected heteronuclear multiple bond H–C correlation experiment (HMBC)[9] (Table 2). The linkage of **B** and **C** was obtained by the coupling of H_{8Me} with C_8,

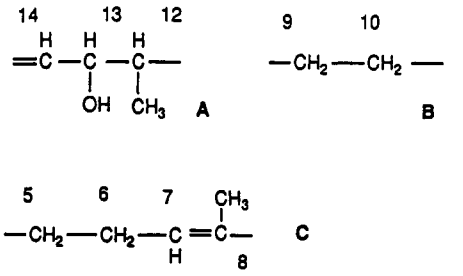
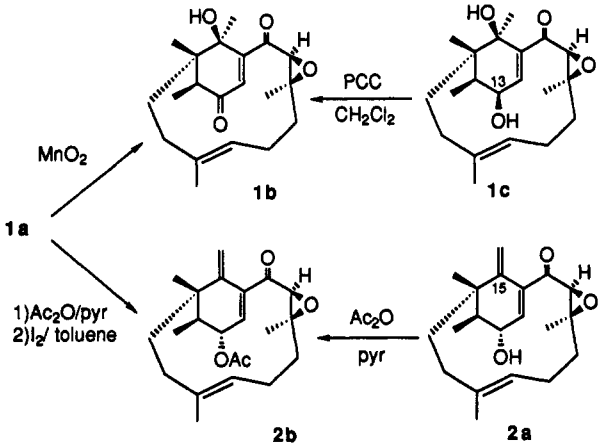


Figure 1.

Scheme 1



C_9, and C_7. The cross peaks of H_{15Me} to C_{15}, C_{11}, C_1 and H_{11Me} to C_{11}, C_{12}, C_{15} confirmed that **A** and three other carbons (C_{11}, C_{15}, C_1) were congruent to a cyclohexene ring. The three-bond cross peaks between H_{11Me} and C_{10} established the attachment of **B** to this ring. The protons of H_{4Me} showed two- and three-bond correlations to C_4, C_3, and C_5. No coupling was observed between H_3 and H_{4Me}. These data suggested the C_3–C_4–C_5 linkage. Insertion of a carbonyl group between C_1 and C_3 was based on the couplings of H_3, H_{14} to C_2 and the UV absorption due to the conjugated enone. The difference NOE experiments allowed for assignment of the relative stereochemistry and conformation; irradiation of H_3 resulted in enhancement of the resonance for H_{15Me}, and irradiation of H_{4Me} enhanced H_{14}, while no enhancement was observed by irradiation at H_3. Similarly, irradiation of $H_{15\text{-}OH}$ enhanced the resonances of H_{13}, H_{11Me}, and H_{12Me}. The NOE interaction between H_{13} and $H_{15\text{-}OH}$ suggested that this ring was in a boat conformation, and the ^{1}H–^{1}H coupling between H_{12} and H_{13} (3.2 Hz) also suggested that these two protons were not trans-axial. Due to the steric hindrance between Me_{11} and Me_{12}, and the strain of a 12-membered ring, this cyclohexene ring was in a pseudo-boat conformation. These data were consistent with the structure **1a** as phomactin B. To confirm the structure, X-ray analysis was attempted on **1b**,[10a] obtained by MnO_2 oxidation of **1a** (Scheme 1). The structure was determined by the direct method (MULTAN 78) and successive block-diagonal least-squares and Fourier synthesis. Parameters

(6) These signals were measured in DMSO-d_6.

(7) (a) Piantin, V.; Sorensen, O. W.; Ernnst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801. (b) Smaka, A. J.; Freedman, R. *J. Magn. Reson.* **1983**, *51*, 169–173. (c) Tance, M.; Sorensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1984**, *51*, 479–485.

(8) (a) Eich, G.; Bodinhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731–3732. (b) Bax, A. D.; Drobny, G. *J. Mag. Reson.* **1982**, *61*, 306–320.

(9) (a) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094. (b) Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *Ibid.* **1986**, *108*, 8056–8063.

(10) (a) **1b**: space group $P2_12_12_1$, a = 15.218(4), b = 8.9213(9), c = 13.671(2), V = 1856.0(4), Z = 4, D_c = 1.19 g/cm^{-3}, ν(Cu Kα) = 6.6 cm^{-1}. (b) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

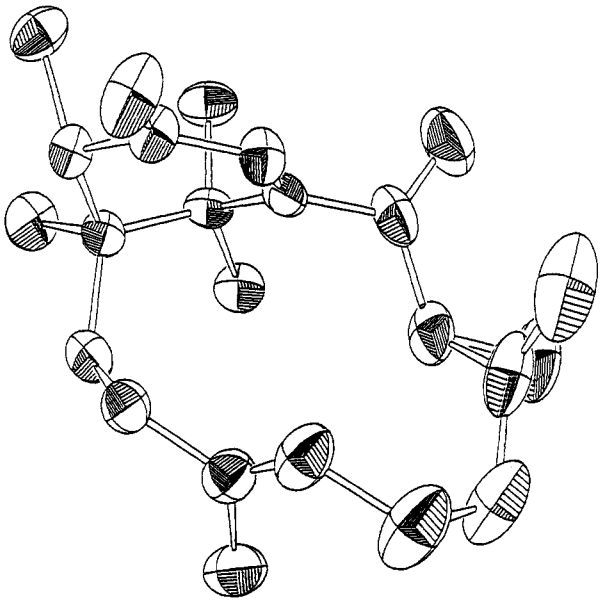


Figure 2. ORTEP drawing of **1b**.

Table 3. ^1H and ^{13}C NMR of Phomactins B_1 and B_2 (CD_3OD)

	phomactin B_1		phomactin B_2	
	^{13}C, ppm	^1H, ppm	^{13}C, ppm	^1H, ppm
1	150.8 (s)		146.6 (s)	
2	202.0 (s)		201.8 (s)	
3	68.6 (d)	3.91 (s)	66.1 (d)	4.12 (s)
4	64.1 (s)		64.7 (s)	
5	39.0 (t)	2.14 (m)	38.4 (t)	2.04 (m)
		1.21 (m)		1.34 (m)
6	24.4 (t)	2.49 (m)	24.5 (t)	2.35 (m)
		2.14 (m)		2.10 (m)
7	121.1 (d)	5.36 (br t, 7.2)	123.7 (d)	5.08 (br t)
8	137.9 (s)		137.9 (s)	
9	33.7 (t)	2.19 (m)	34.7 (t)	2.16 (m)
		2.19 (m)		2.16 (m)
10	34.3 (t)	2.10 (m)	35.3 (t)	1.93 (br d, 13.7)
		1.43 (m)		1.56 (m)
11	47.1 (s)		42.4 (s)	
12	47.8 (d)	1.86 (qd, 7.8, 6.8)	45.9 (d)	1.72 (qd, 7.3, 2.4)
13	68.9 (d)	4.72 (dd, 6.8, 2.9)	72.0 (d)	3.98 (dd, 2.4, 3.9)
14	135.0 (d)	5.74 (d, 2.9)	134.3 (d)	6.00 (d, 3.9)
15	74.0 (s)		142.4 (s)	
4Me	12.9 (q)	1.22 (s)	14.7 (q)	1.08 (s)
8Me	15.6 (q)	1.63 (s)	15.4 (q)	1.58 (s)
11Me	20.1 (q)	1.19 (s)	16.6 (q)	1.12 (s)
12Me	18.0 (q)	1.16 (d, 7.8)	23.8 (q)	0.83 (d, 7.3)
15Me	25.6 (q)	1.47 (s)	117.0 (t)	5.34 (d, 1.5)
				5.26 (s)

were refined by using anisotropic temperature factors to $R = 0.040$ for 1280 reflections [$|F_o| > 3\sigma(F_o)$].[10b]

The X-ray analysis revealed a further structural feature, in that the enone conjugation was twisted; the dihedral angle between the carbonyl and the $\Delta^{1,14}$ double bond was 94°. This accounted for the low ϵ in the UV,[11] and an unusual ^{13}C NMR assignment at C_1 (δ 147.2) and C_{14} (δ 135.6).

Phomactin B1 (**1c**) was obtained as colorless crystals. In ^1H NMR and ^{13}C NMR (Table 3), H_{13} (δ 4.72) differed from that of **1a** (δ 4.12) and H_{13} and H_{12} showed the coupling constant 6.8 Hz ($J = 3.2$ Hz in **1a**), compatible with the cis configuration of H_{12} and H_{13}. **1c** was therefore an epimer of **1a** at C_{13}. This structure was confirmed by

(11) Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: New York, 1964; p 55.

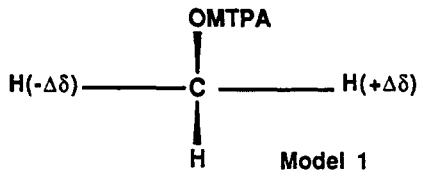


Figure 3.

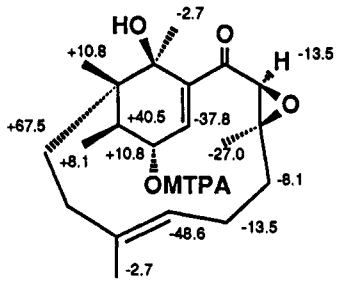


Figure 4. $\Delta\delta$ Values shown in hertz (270 MHz).

PCC oxidation of **1c** to **1b** (Scheme 1). Phomactin B2 (**2a**) was obtained as a colorless oil. The ^1H and ^{13}C NMR (Table 3) contained signals characteristic of an exocyclic methylene [δ_H 5.34 (1H, d, $J = 1.5$ Hz), 5.26 (1H, s); δ_C 117.0 (t), 142.4 (s)] and differed from **1a** only at C_{15} and C_{15Me}. It was therefore postulated that phomactin B2 had the structure **2a**, which was confirmed by chemical conversion; phomactin B acetate was dehydrated to **2b** with iodine. **2b** was also obtained by the acetylation of **2a** (Scheme 1). The structure of phomactin B2 was therefore determined to be **2a**.

To ascertaiin the absolute stereochemistry of these compounds, we applied two methods: the advanced Mosher's method and circular dichroism.[12]

Initially, the advanced Mosher's method[13] was applied to **1a**. **1a** was converted to (+)-(*R*)- and (−)-(*S*)-MTPA esters, **1d** and **1e**, respectively. All the proton signals of each compound were assigned, and $\Delta\delta$ [$\delta(-) - \delta(+)$] was calculated for each proton (Figure 4). The protons with positive $\Delta\delta$ were designated as right hand side and conversely that of negative $\Delta\delta$ on the left hand side, in model 1 (Figure 3). In accordance with our predictions all the assigned protons were actually found on the right and left sides of the MTPA planes in the model 1. The $\Delta\delta$ was proportional to the distance from MTPA planes; X-ray analysis of **1b** showed that H_7, H_{12}, H_{14}, and H_{4Me} were closer to this plane than H_{8Me}, H_{11Me}, H_{15Me}, and H_3, respectively. These conditions were satisfactory enough to apply the model 1, and we determined the absolute stereochemistry of phomactin B to be **1a**.

For the circular dichroism method, the CD[14] between the diene and the benzoate of **2d** was applied.[15] However **2d** itself bears a π–π^* transition (230–280 nm) due to the twisted diene [C14–C1–C15–C(15-*exo*methylene)]. We

(12) Since the hydroxy at C_{13} could not react with the anhydride easily, the Horeau method (*Tetrahedron Lett.* **1952**, 965–969.) could not be applied.

(13) (a) Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731–4734. (b) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Ibid.* **1989**, *30*, 3147–3150. (c) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

(14) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy; Exiton Coupling in Organic Spectrometry*; University Science Books; Mill Valley, CA, 1983.

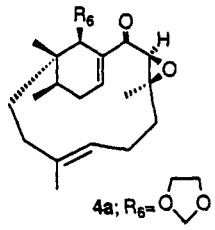
(15) The twisted enone (O–C2–C1–C14) conjugation exhibits a π–π^* transition (K-band) making the CD spectral interpretation somewhat complicated. To observe the circular dichroism between the diene and the benzoate directly, we reduced the ketone to hydroxy.

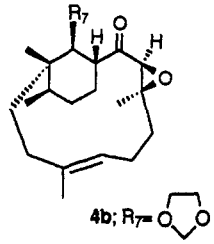
therefore prepared **2c** and observed the CD spectrum of [**2d**–**2c**] to erase the Cotton effect of the diene. Since a positive Cotton effect [λ ext 237 nm ($\Delta\epsilon$ +33.0), 220 nm ($\Delta\epsilon$ −4.6)] was observed (Figure 5), the absolute configuration of **2c** was determined.

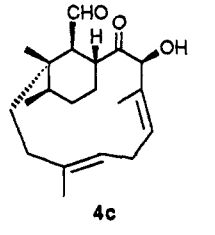
On the basis of these spectral data, phomactin B was determined to be (3*S*,4*R*,11*S*,12*S*,13*R*,15*R*)-13,15-dihydroxy-3,4-epoxy-2-oxo-4,8,11,12,15-pentamethylbicyclo-[9.3.1]-7(*E*),14-pentadecadiene. Phomactins B1 and B2 were also determined to be, respectively, (3*S*,4*R*,11*S*,12*S*,-13*S*,15*R*)-13,15-dihydroxy-3,4-epoxy-2-oxo-4,8,11,12,15-pentamethylbicyclo[9.3.1.]-7(*E*),14-pentadecadiene and (3*S*,4*R*,11*S*,12*R*,13*R*)-13-hydroxy-3,4-epoxy-2-oxo-4,8,11,-12-tetramethyl-15-methylenebicyclo[9.3.1]-7(*E*),14-pentadecadiene.

Phomactin C (**4**) was obtained as colorless crystals (mp 204–205 °C). UV and ^{1}H and ^{13}C NMR data indicated that phomactin C was identical with Sch 47918, whose structure was determined by X-ray analysis.

Phomactin D (**5**) was obtained as colorless crystals (mp 97–98 °C). The molecular formula $C_{20}H_{30}O_3$ was determined by HREIMS (*m*/*z* 318.22078; Δ −0.18 mmu). DQF COSY and HMBC experiments showed that **5** had the same skeletal framework as **4**. One double bond signal [δ_C 141.6 (s), 127.9 (d), δ_H 5.65 (1H, dd *J* = 7.5, 6.4 Hz)] in the ^{1}H and ^{13}C NMR, and the UV end absorption were compatible with the structure **5**. To confirm the structure, **4** was converted to **5**; the ethylene ketal **4a** of **4** underwent a 1,4-reduction by using DIBAH, to give **4b**. However HCl treatment of **4b** gave the undesired main product **4c**. Another route was then employed; **4** was reduced by DIBALH to give **4d**. **4d** was oxidized by PDC to give **5** (total yield 51%). Configuration of **5** was derived from the NOE interaction between H_1 and H_{11Me}, H_{15CHO}, which established the relative stereochemistry of C_1 to be 1*R*.







The PAF antagonistic activities of **1a**, **1c**, **2a**, **4**, and **5** are listed in Table 4. Phomactin D inhibited the binding of PAF to its receptors and PAF-induced platelet aggregation with IC_{50} of 2.8 × 10^{-7} M and 8.0 × 10^{-7} M, respectively, while other compounds antagonized the PAF action at higher concentrations. These data suggested that the conformation of the bicyclic ring system and the substitution pattern of hydroxy groups thereon have significant forbearance toward specific binding. The structure–activity relationship of natural phomactins and the absolute stereochemistry of phomactin C and D are being investigated. Their derivatives have been extensively studied and will be published elsewhere.

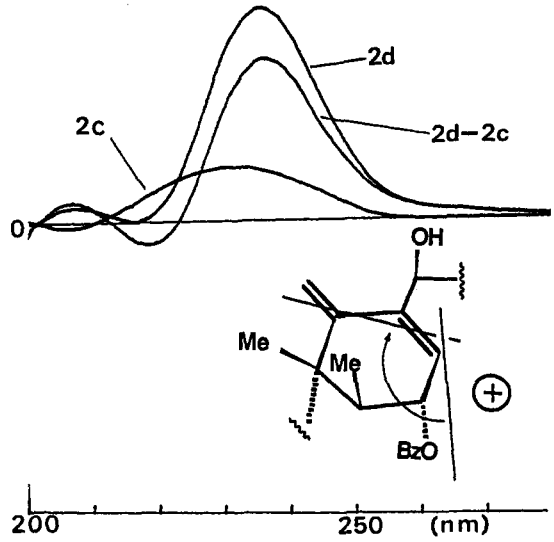


Figure 5.

Table 4. Biological Activities of Phomactins

	platelet aggregation: IC_{50} (μM)	PAF binding: IC_{50} (μM)
1a	17.0	>47.9
1c	9.8	20.0
2a	1.6	>22.1
4	6.4	63.0
5	0.80	0.12

Experimental Section

Isolation. The culture broth (600 L) was filtered, and the filtrate was extracted with ethyl acetate (800 L). The EtOAc layer was washed with H_2O and evaporated to dryness under reduced pressure, to give an oily substance (483.3 g). The oily substance was twice fractionated by silica gel column chromatography (2.0 kg, hexane–EtOAc 4:6), to give two fractions Fr 1 (15.59 g) and Fr 2 (36.9 g). Fr 1 was dissolved in MeOH, and the filtrate was subjected to silica gel column chromatography (100 g, acetone–CH_2Cl_2 2:98), to give **4** (1.008 g). The filtrate was subjected to silica gel column chromatography (300 g, acetone–CH_2Cl_2 5:95–1:9), to give **5** (84.2 mg). The other combined fractions were subjected to reversed-phase column chromatography (Lobar RP-8 B size, Merck; 85% aqueous MeOH) to give **2a** (677.0 mg). Fr 2 was chromatographed on a silica gel column (400 g, gradient hexane–EtOAc system) to give active fractions Fr-2-1 (11.38 g) and Fr 2-2 (16.54 g). Fr 2-1 was subjected to reversed-phase column (system 500 HPLC, 80% MeOH) to give **3**. Fr 2-2 was chromatographed on silica gel column (600 g, EtOAc-*i*-PrOH) to give Fr 2-2-1 (10.49 g) and Fr 2-2-2 (1.68 g), and Fr 2-2-1 was subjected to silica gel column (300 g, $CHCl_3$–MeOH 95:5) to give **1a** (2103.0 mg). Fr 2-2-2 was subjected to reversed-phase column (Lobar RP-8 B size 75 % aqueous MeOH) to give **1c** (46.0 mg). These fermentation and isolations were carried out several times to afford **3** for the reaction below.

Phomactin B (1a): mp 180–182 °C; $[\alpha]_D$ +146° (*c* = 0.75 $CHCl_3$); UV (EtOH) λ_{max} 240 nm (ϵ 3100); EIMS (*m*/*z*) 334, 316, 298, 288, 255, 246, 219, 203, 189, 177, 163, 149, 135, 121, 107, 91, 80, 67, 55, 40; IR (KBr) ν_{max} 3420, 3380, 1670, 1630, 1460, 1390, 1350, 1200, 1080, 1000, 900, 810 cm^{-1}. ^{1}H and ^{13}C NMR are listed in Tables 1 and 2.

Phomactin B1 (1c): mp 203–205 °C; $[\alpha]_D$ +167.3° (*c* = 1.0 $CHCl_3$); HREIMS (*m*/*z* 334.21086; Δ −3.5 mmu); UV (EtOH) λ_{max} 235 nm (ϵ 3600); EIMS (*m*/*z*) 334, 316, 301, 273, 255, 247, 180, 137, 127, 121, 109, 95, 81, 69, 55, 43; IR (KBr) ν_{max} 3450, 3370, 2940, 1670, 1380, 1210, 1090, 1040, 910, 850 cm^{-1}. ^{1}H and ^{13}C NMR are listed in Table 3.

Phomactin B2 (2a): oil; $[\alpha]_D$ +173° (c = 5.0 $CHCl_3$); HREIMS (m/z 316.20340; Δ −0.4 mmu); UV (EtOH) λ_{max} 262 nm (ε 3600), 221 nm (ε 8700); EIMS (m/z) 316, 301, 273, 248, 231, 217, 203, 189, 175, 165, 149, 133, 121, 109, 91, 67, 55; IR ($CHCl_3$) ν_{max} 3500, 2950, 1690, 1610, 1460, 1390, 1220, 1000, 910. ^1H and ^{13}C NMR are listed in Table 3.

Phomactin D (5): mp 97–98 °C; $[\alpha]_D$ +114.3° (c = 1.0 $CHCl_3$); UV end absorption; EIMS (m/z) 318, 301, 275, 249, 233, 219, 203, 175, 161, 137, 121, 95, 81, 67; IR (KBr) ν_{max} 2960, 1710, 1460, 1450, 1400, 1390, 1080, 830 cm^{-1}; ^1H NMR (CD_3OD) δ 10.13 (1H, s), 5.65 (1H, dd, J = 7.5, 6.4 Hz), 4.25 (1H, d, J = 11.6 Hz), 3.19 (1H, s), 2.60–2.73 (2H, m), 2.00–2.32 (6H, m), 1.75 (3H, s), 1.62–1.73 (3H, m), 1.31–1.60 (1H, m), 1.20–1.30 (2H, m), 1.18 (3H, s), 0.89 (3H, d, J = 6.9 Hz), 0.80 (3H, s); ^{13}C NMR (CD_3OD) δ 208.0 (s), 207.4 (d), 141.6 (s), 127.9 (d), 66.9 (d), 64.6 (s), 53.8 (d), 46.8 (d), 39.7 (s), 37.7 (t), 36.5 (d), 36.2 (t), 32.3 (t), 29.4 (t), 29.3 (t), 24.3 (t), 18.4 (q), 17.4 (q), 16.5 (q), 14.7 (q). Anal. Calcd: C, 75.43%; H, 9.50%. Found: C, 75.20%; H, 9.50%.

Oxidation of 1a: To a solution of **1a** (50.0 mg) in CH_2Cl_2 (2.0 mL) was added MnO_2 (50.0 mg). The mixture was stirred at room temperature for 2 h and was then evaporated to dryness. The residue was subjected to silica gel column chromatography (hexane–EtOAc 6:4) to give a crystal **1b** (38.0 mg). The sample for X-ray analysis was recrystallized from hexane–ether: mp 140–143 °C; HREIMS (m/z 332.19941; Δ+0.6 mmu); UV (EtOH) λ_{max} 231 nm (ε 5400); EIMS (m/z) 332, 299, 289, 271, 243, 231, 178, 137, 125, 109, 95, 69, 55, 43; IR (KBr) ν_{max} 3420, 3000, 2960, 1710, 1660, 1450, 1380, 1270, 1100, 910, 820 cm^{-1}; ^1H NMR (CD_3OD) δ 5.63 (1H, d, J = 1.0 Hz), 5.07 (1H, br t, J = 7.3 Hz), 3.80 (1H, s), 1.67–2.37 (8H, m), 1.65 (3H, s), 1.49 (3H, s), 1.42–1.47 (1H, m), 1.38 (3H, d, J = 7.8 Hz), 1.27 (3H, s), 1.19 (3H, s); ^{13}C NMR (CD_3OD) δ 206.4 (s), 200.7 (s), 164.9 (s), 138.0 (s), 126.0 (d), 125.2 (d), 73.4 (s), 68.7 (d), 65.1 (s), 53.4 (d), 46.6 (s), 38.5 (t), 37.3 (t), 36.0 (t), 24.3 (t), 23.7 (q), 22.6 (q), 18.1 (q), 16.3 (q), 16.2 (q).

Oxidation of 1c: To a solution of **1c** (8.0 mg) in CH_2Cl_2 (3.0 mL) was added PCC (10.0 mg). The mixture was stirred at room temperature for 1 h and was then evaporated to dryness. The residue was subjected to silica gel column chromatography (hexane–EtOAc 1:1) to give **1b** (6.8 mg).

Acetylation and dehydration of 1a: To a solution of **1a** (103.0 mg) in pyridine (3.0 mL) was added acetic anhydride (0.5 mL). The mixture was stirred at room temperature for 2 h and then was evaporated to dryness. The residue was dissolved in toluene (5.0 mL) and was refluxed with a catalytic amount of I_2 for 1.5 h. The solvent was evaporated to dryness. The residue was subjected to silica gel column chromatography (hexane–EtOAc 92:8) to give **2b** (81.6 mg): oil; HREIMS (m/z 358.21467; Δ+0.2 mmu); UV (EtOH) λ_{max} 215 nm (ε 9700), EIMS (m/z) 358, 316, 288, 255, 201, 173, 165, 147, 133, 119, 91, 81, 55, 43; IR ($CHCl_3$) ν_{max} 3550, 2950, 1730, 1690, 1630, 1460, 1380, 1220, 1020, 920 cm^{-1}; ^1H NMR (CD_3OD) δ 5.97 (1H, d, J = 4.4 Hz), 5.41 (1H, s), 5.40 (1H, s), 5.08 (1H, br s), 5.05 (1H, dd, J = 4.4, 1.5 Hz), 4.14 (1H, s), 2.09–2.32 (5H, m), 2.06 (3H, s), 1.60 (3H, s), 1.61–1.98 (2H, m), 1.16–1.32 (2H, m), 1.12 (3H, s), 1.09 (3H, s), 0.86 (3H, d, J = 7.3 Hz); ^{13}C NMR (CD_3OD) δ 200.7 (s), 171.8 (s), 145.0 (s), 144.6 (s), 137.9 (s), 129.2 (d), 123.4 (d), 119.0 (t), 74.4 (d), 66.3 (d), 64.9 (s), 44.5 (d), 42.7 (s), 38.9 (t), 35.1 (t), 34.5 (t), 24.7 (t), 22.7 (q), 21.1 (q), 16.4 (q), 14.8 (2C, q).

Acetylation of 2a: To a solution of **2a** (15.0 mg) in pyridine (3.0 mL) was added acetic anhydride (0.5 mL). The mixture was stirred at room temperature for 2 h and was then evaporated to give **2b** (13.2 mg).

1d: To a solution of **1a** (8.0 mg) in pyridine (1.0 mL) was added (+)-(*R*)-MTPA (30 μL). The mixture was stirred at room temperature for 30 min and was then evaporated to dryness. The residue was subjected to silica gel column chromatography (hexane–EtOAc 8:2) to give **1d** (13.3 mg): mp 154–155 °C; HREIMS (m/z 550.25053; Δ −3.6 mmu); EIMS (m/z) 550, 532, 426, 413, 399, 343, 189, 147, 137, 119, 109, 81, 69, 43; IR (KBr) ν_{max} 3510, 2950, 2600, 1750, 1700, 1450, 1380, 1270, 1240, 1160, 1020, 910 cm^{-1}; ^1H NMR (CD_3OD) δ 7.52 (2H, m), 7.42 (3H, m), 5.73 (1H, d, J = 3.4 Hz), 5.41 (1H, dd, J = 3.4, 1.5 Hz), 5.15 (1H, br t, J = 7.1 Hz), 3.83 (1H, s), 3.54 (3H, s), 2.39 (1H, m), 1.93–2.17 (5H, m), 1.63 (1H, dq, J = 1.5, 7.8 Hz), 1.54 (3H, s), 1.46 (3H, s), 1.32 (3H, d, J = 7.8 Hz), 1.27 (1H, m), 1.16 (3H, s), 1.11 (3H, s), 0.95 (1H, m); ^{13}C NMR (CD_3OD) δ 202.0 (s), 166.9 (s), 153.5 (s), 138.0 (s), 133.5 (s), 130.8 (d), 129.6 (3C, d), 128.9 (d), 128.4 (d), 124.8 (q), 121.3 (d), 86.1 (q), 77.9 (d), 74.3 (s), 68.5 (d), 64.5 (s), 56.1 (q), 46.6 (d), 42.9 (s), 38.8 (t), 36.9 (t), 34.0 (t), 24.7 (q), 24.2 (t), 19.2 (q), 19.1 (q), 17.5 (q), 15.4 (q). Anal. Calcd: C, 65.44%; H, 6.77%; F, 10.35%. Found: C, 65.77%; H, 6.79%; F, 10.11%.

1e: To a solution of **1a** (12.2 mg) in pyridine (1.0 mL) was added (−)-(*S*)-MTPA (30 μL). The mixture was stirred at room temperature for 30 min and was then evaporated to dryness. The residue was subjected to silica gel column chromatography (hexane–EtOAc 8:2) to give **1e** (14.0 mg): oil; HREIMS (m/z 550.25230; Δ −1.8 mmu); EIMS (m/z) 532, 413, 399, 383, 189, 177, 147, 137, 119, 109, 95, 81, 69, 55, 43; IR (CH_2Cl_2) 3450, 2950, 1740, 1690, 1650, 1450, 1390, 1220, 1180, 1120, 1080, 1010, 900 cm^{-1}; ^1H NMR (CD_3OD) δ 7.53 (2H, m), 7.42 (3H, m), 5.56 (1H, d, J = 3.4 Hz), 5.45 (1H, dd, J = 3.4, 2.0 Hz), 4.97 (1H, br t, J = 7.3 Hz), 3.78 (1H, s), 3.54 (3H, s), 2.34 (1H, m), 1.93–2.13 (5H, m), 1.78 (1H, dq, J = 2.0, 7.3 Hz), 1.53 (3H, s), 1.45 (3H, s), 1.35 (3H, d, J = 7.3 Hz), 1.24 (1H, m), 1.20 (1H, m), 1.15 (3H, s), 1.06 (3H, s); ^{13}C NMR (CD_3OD) δ 201.9 (s), 166.9 (s), 153.0 (s), 137.8 (s), 133.5 (s), 130.7 (d), 129.5 (2C, d), 128.5 (d), 128.3 (2C, d), 124.8 (q), 121.4 (d), 85.8 (q), 77.7 (d), 74.3 (s), 68.3 (d), 64.5 (s), 56.0 (q), 46.2 (d), 42.9 (s), 38.7 (t), 37.1 (t), 34.1 (t), 24.3 (q), 24.0 (t), 19.5 (2C, q), 17.3 (q), 15.4 (q). Anal. Calcd: C, 65.44%; H, 6.77%; F, 10.35%. Found: C, 65.67%; H, 6.74%; F, 10.09%.

2c: To a solution of **2a** (20 mg) in EtOH (5.0 mL) was added $NaBH_4$ (5.0 mg). The mixture was stirred at room temperature for 1 h and was then evaporated to dryness. The residue was subjected to silica gel column chromatography (CH_2Cl_2–acetone 95:5) to give **2c** (17.2 mg): oil; HREIMS (m/z 318.2185; Δ −1.0 mmu); EIMS (m/z) 318, 300, 272, 257, 243, 215, 201, 173, 166, 149, 135, 121, 91, 81, 67, 43; IR ($CHCl_3$) ν_{max} 3500, 2900, 1670, 1600, 1450, 1380, 1220, 1000, 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.04 (1H, d, J = 4.2 Hz), 5.27 (1H, s), 5.18 (1H, dt, J = 4.1, 1.9 Hz), 5.06 (1H, d, J = 1.5 Hz), 4.90 (1H, br t, J = 6.5 Hz), 4.04 (1H, ddd, J = 4.2, 2.6, 2.1 Hz), 3.08 (1H, J = 4.1 Hz), 1.92–2.18 (6H, m), 1.55–1.79 (3H, m), 1.52 (3H, s), 1.39 (3H, s), 1.17–1.26 (2H, m), 1.01 (3H, s), 0.84 (3H, d, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 145.0 (s), 137.0 (s), 134.0 (s), 123.5 (d), 121.6 (d), 110.8 (t), 71.3 (d), 65.3 (d), 64.4 (d), 62.8 (s), 44.6 (d), 42.0 (s), 38.9 (t), 35.2 (t), 34.4 (t), 23.9 (t), 23.4 (q), 16.5 (q), 15.9 (q), 14.6 (q).

2d: To a solution of **2a** (50.0 mg) in pyridine (3.0 mL) was added benzoyl chloride (30 mg). The mixture was stirred at room temperature for 5 h. After usual workup, the residue was treated with $NaBH_4$ (20.0 mg) in EtOH (5.0 mL) at room temperature. After 1 h, EtOH was evaporated, and the residue was subjected to silica gel column chromatography (hexane–EtOAc 85:15) to give **2d** (48.7 mg): oil; HREIMS (m/z 422.2451; Δ −0.6 mmu); EIMS (m/z) 422, 404, 371, 355, 317, 300, 270, 215, 201, 162, 149, 121, 105, 77, 55; IR ($CHCl_3$) ν_{max} 3500, 2900, 1700, 1600, 1450, 1310, 1270, 1010, 950 cm^{-1}; ^1H NMR (CD_3OD) δ 8.10 (2H, dd, J = 7.6, 1.2 Hz), 7.68 (1H, dd, J = 7.6, 1.2 Hz), 7.55 (2H, t, J = 7.6 Hz), 6.19 (1H, d, J = 4.7 Hz), 5.57 (1H, s), 5.41 (1H, dd, J = 4.9, 2.8 Hz), 5.31 (1H, d, J = 3.9 Hz), 5.27 (1H, s), 5.09 (1H, br t, J = 6.6 Hz), 3.12 (1H, d, J = 3.9 Hz), 2.40–2.50 (1H, m), 1.19–2.19 (6H, m), 1.51–1.90 (1H, m), 1.64 (3H, s), 1.44 (3H, s), 1.18–1.20 (1H, m), 1.16 (3H, s), 1.01 (3H, d, J = 7.2 Hz); ^{13}C NMR (CD_3OD) δ 165.4 (s), 144.1 (s), 138.2 (s), 135.7 (s), 132.4 (d), 129.9 (s), 128.5 (2C, d), 127.7 (2C, d), 120.5 (d), 117.7 (d), 111.6 (t), 73.5 (d), 64.6 (d), 63.4 (d), 61.0 (s), 42.3 (d), 41.2 (s), 38.6 (t), 34.1 (t), 32.8 (t), 22.5 (t), 21.9 (q), 15.1 (q), 14.0 (q), 13.2 (q).

Conversion of 4 to 4a: To a solution of a catalytic amount of TsOH and ethylene glycol (100 μL) in benzene (10.0 mL) was added **3** in benzene (10.0 mL). The mixture was refluxed with a Dean–Stark apparatus for 20 min. This solution was then poured onto saturated $NaHCO_3$ solution (50.0 mL). After usual workup the organic layer was evaporated to give crude **4a**. **4a** (90.2 mg) crystallized from hexane–CH_2Cl_2: mp 157–158 °C; UV λ_{max} 240 nm (ε 2500); EIMS (m/z) 360, 345, 315, 302, 287, 270, 255, 207, 175, 163, 135, 105, 91, 73, 45; IR (KBr) ν_{max} 2970, 2900, 1690, 1620, 1440, 1390, 1190, 1120, 1040, 1030, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.61 (1H, br s), 5.20 (1H, br d, J = 8.8 Hz), 5.04 (1H, d, J = 5.4 Hz), 3.95 (1H, s), 3.72–4.05 (4H, m), 3.23 (1H, br s), 2.52–2.61 (1H, m), 2.12–2.34 (5H, m), 1.99 (1H, dd, J = 5.0, 2.0 Hz); 1.68 (1H, dd, J = 5.6, 14.0 Hz), 1.60 (3H, d, J = 6.4 Hz), 1.50 (1H, m), 1.16–1.27 (2H, m), 1.14 (3H, s), 1.09 (3H, s), 1.00 (3H, d, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 196.3 (s), 137.8 (s), 137.3 (s),

134.1 (d), 123.9 (d), 103.7 (d), 65.1 (d), 65.0 (t), 64.5 (t), 62.4 (s), 42.6 (t), 38.4 (d), 37.7 (s), 36.6 (d), 35.4 (t), 34.6 (t), 31.0 (t), 24.4 (t), 21.3 (q), 17.6 (q), 16.0 (q), 14.5 (q). Anal. Calcd: C, 73.30%; H, 8.95%. Found: C, 73.07%; H, 9.13%.

Conversion of 4a to 4b: To the cooled solution (−60 °C) for **4a** (125.0 mg) in CH_2Cl_2 was added DIBALH (1 M solution; 520 μL). The solution was stirred at −60 °C for 30 min and then quenched with saturated $NaHCO_3$ solution (10.0 mL) under stirring at room temperature for 30 min. This solution was then filtered, and the organic layer was evaporated to dryness. The residue was subjected to silica gel column chromatography (CH_2Cl_2–acetone 98:2) to give **4b** (88.0 mg): mp 146 °C; UV end absorption; EIMS (*m/z*) 362, 263, 238, 209, 181, 167, 133, 121, 109, 73, 55, 45. IR (KBr) ν_{max} 2980, 2940, 1710, 1380, 1140, 1080, 1020, 960, 820, 730, 590 cm^{-1}; ^1H NMR (CD_3OD) δ 5.25 (1H, br d, *J* = 6.9 Hz), 5.10 (1H, d, *J* = 1.2 Hz), 4.02 (1H, s), 3.80–3.91 (2H, m), 3.62–3.74 (2H, m), 3.37 (1H, d, *J* = 11.5 Hz), 2.62–2.71 (1H, m), 2.51 (1H, td, *J* = 13.8, 2.6 Hz), 1.89–2.09 (7H, m), 1.68 (3H, s), 1.22–1.60 (5H, m), 1.15 (3H, s), 0.87 (3H, d, *J* = 6.9 Hz), 0.77 (3H, s); ^{13}C NMR (CD_3OD) δ 209.6 (s), 138.5 (s), 129.9 (d), 105.5 (d), 66.3 (t), 65.0 (t), 64.0 (s), 63.0 (d), 48.0 (d), 43.0 (d), 38.8 (s), 36.7 (t), 36.3 (d), 35.2 (t), 33.0 (t), 30.1 (t), 29.1 (t), 23.7 (t), 20.5 (q), 17.8 (q), 15.9 (q), 15.5 (q). Anal. Calcd: C, 72.89%; H, 9.45%. Found: C, 72.72%; H, 9.54%.

Conversion of 4b to 4c and 5: To a solution of **4b** (17.0 mg) in THF (3.0 mL) was added HCl (700 μL) in H_2O (2.0 mL). This solution was stirred at room temperature for 5 h. To this solution were added EtOAc (10.0 mL) and saturated $NaHCO_3$ solution (10.0 mL), and the organic layer was evaporated to dryness. The residue was subjected to silica gel column chromatography (CH_2Cl_2–acetone 98:2) to give **4c** (6.0 mg) and **5** (7.8 mg).

4c: mp 113 °C; EIMS (*m/z*) 318, 300, 285, 272, 236, 235, 217, 189, 175, 149, 133, 109, 95, 84, 81, 41; IR (KBr) ν_{max} 3460, 2970, 2940, 1700, 1450, 1380, 1240, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 10.07 (1H, s), 5.37 (1H, br t, *J* = 8.1 Hz), 5.33 (1H, br d, *J* = 10.1 Hz), 4.79 (1H, s), 4.13 (1H, d, *J* = 11.7 Hz), 3.18 (1H, dt, *J* = 14.9, 10.0 Hz), 2.53–2.65 (3H, m), 1.99–2.31 (3H, m), 1.74 (3H, s), 1.52–1.71 (2H, m), 1.50 (3H, s), 1.19–1.46 (3H, m), 0.87 (3H, d, *J* = 6.8 Hz), 0.78 (3H, s); ^{13}C NMR (CD_3OD) δ 213.2 (s), 207.3 (d), 136.7 (s), 136.3 (s), 130.1 (d), 127.8 (d), 72.3 (d), 55.0 (d), 50.2 (d), 40.6 (s), 36.5 (t), 35.7 (d), 32.1 (t), 30.7 (t), 30.6 (t), 28.2 (t), 18.9 (q), 17.7 (q), 17.1 (q), 15.2 (q). Anal. Calcd: C, 75.43%; H, 9.50%. Found: C, 75.10%; H, 9.67%.

4d: To a solution of **3** (5.0 g) in CH_2Cl_2 (150.0 mL) was added DIBALH (1 M solution in CH_2Cl_2; 50.0 mL) at −60 °C, and then the mixture was cooled to −70 °C and stirred for 40 min. To this solution was added saturated $NaHCO_3$ solution. The mixture was then stirred at room temperature for 30 min and was filtered with Celite. The organic layer was evaporated to dryness. The residue was subjected to silica gel column chromatography (CH_2Cl_2–acetone 9:1) to give **4d** (2.8 g): mp 166 °C; EIMS (*m/z*) 320, 302, 289, 262, 250, 221, 207, 189, 161, 151, 135, 123, 95, 81, 55, 41; IR (KBr) ν_{max} 3540, 2960, 2930, 1700, 1440, 1380, 1310, 1060, 1040, 970, 800, 660 cm^{-1}; ^1H NMR (CD_3OD) δ 5.25 (1H, br d, *J* = 7.9 Hz), 4.14 (1H, s), 3.95 (1H, dd, *J* = 9.9, 3.4 Hz), 3.38 (1H, dd, *J* = 9.9, 10.7 Hz), 3.15 (1H, td, *J* = 10.7, 3.4 Hz), 2.50 (1H, td, *J* = 13.6, 2.4 Hz), 2.27–2.34 (1H, m), 1.84–2.12 (7H, m), 1.74 (3H, s), 1.54–1.60 (1H, m), 1.35–1.47 (1H, m), 1.17–1.34 (3H, m), 1.16 (3H, s), 0.86 (3H, d, *J* = 8.1 Hz), 0.62 (3H, s); ^{13}C NMR (CD_3OD) δ 210.3 (s), 139.4 (s), 128.9 (d), 65.9 (t), 64.2 (d), 64.2 (s), 54.7 (d), 41.0 (d), 38.9 (s), 36.8 (t), 36.5 (d), 35.8 (t), 32.5 (t), 30.6 (t), 29.4 (t), 23.9 (t), 20.5 (q), 16.1 (q), 15.7 (q), 15.4 (q). Anal. Calcd: C, 74.96%; H, 10.07%. Found: C, 74.90%; H, 10.27%.

Conversion of 4d to 5: To a solution of **4d** (11.0 mg) in CH_2Cl_2 (3.0 mL) were added molecular sieves (500 mg) and PDC (32.5 mg). The mixture was stirred at room temperature for 40 min and then was evaporated to dryness. The residue was subjected to silica gel column chromatography (hexane–EtOAc 8:2) to give **5** (10.0 mg).

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Supplementary Material Available: Copies of ^1H NMR spectra (28 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm verison of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.