

Phorbasins G—I: Three New Diterpenoids from the Sponge *Phorbas gukulensis*

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Received April 7, 2008; accepted May 19, 2008; published online May 26, 2008

Phorbasins G—I (1–3) were isolated from the sponge *Phorbas gukulensis* collected from Gagu-Do, Korea. The complete structure of the compounds was determined by NMR and MS spectroscopy, along with chemical reaction. Phorbasin G (1) was found to be a diterpene possessing a taurine residue.

Key words phorbasin G; phorbasin H; phorbasin I; *Phorbas gukulensis*; taurine residue; 2 dimensional NMR

Biologically active compounds with unusual functional groups have been isolated from marine sponges over the last four decades.¹⁾ In our search for bioactive natural products from marine sponges, three new diterpenoids were isolated from the extract of the sponge *Phorbas gukulensis* with the cytotoxic gagunin A–D compounds reported previously.²⁾ In the literature survey, metabolites from the genus *Phorbas* were reported to feature heterocyclic phorbaxozoles,³⁾ phorbazoles,⁴⁾ phobasterones,⁵⁾ and diterpene phorbasins.^{6,7)} Though we could not isolate these compounds from our specimen, our three compounds are structural analogs with skeletons similar to diterpene phorbasins and are characterized by modified functional groups. Here we describe the isolation and structure determination of the three diterpene phorbasins G (1), H (2) and I (3).

Phorbasin G (1) was isolated in a yellowish gum and determined to have a molecular formula of C₂₂H₃₆NO₄Na on the basis of high resolution FAB-MS ([M+Na]⁺ peak at *m/z* 456.2159). An FAB tandem MS spectrum of 1 also showed intense fragment ion peaks at *m/z* 372, 319, 286, 224, 169, 140, and 126. The IR spectrum exhibited strong characteristic absorption bands at 1210 cm⁻¹ (S=O stretching), 1039 cm⁻¹ (asymmetric O=S=O stretching), as well as a peak at 1637 cm⁻¹ (C=O stretching). This suggested the presence of a sulfonic acid salt, together with a fragment ion at *m/z* 126 [SO₃Na]⁺ in the positive FAB tandem MS. The ¹³C-NMR spectrum displayed 20 distinct signals: four methyls, six methylenes, seven methines, and three quaternary carbons by an analysis of both the edited HSQC spectrum and the integration of the ¹H-NMR spectrum. Two of these were assigned to degenerate methylenes at δ 32.0 and 30.7, which positions were readily recognized by the doubled integration ratio of their corresponding protons. And 1 had three double bonds (δ 141.2, 139.4, 132.1, 126.5, 125.8, and 124.6) and a carbonyl group (δ 178.8).

From a detailed analysis of the ¹H–¹H COSY and edited HSQC spectra, in conjunction with a gradient HMBC spectrum, 1 was found to contain a diterpene moiety composed of three substructures: 2,6-dimethylundeca-2,7,9-triene, 1,4-disubstituted cyclohexane, and a carbonyl group. The linkage between the linear chain and a symmetrical cyclohexane was revealed by the HMBC correlations from the methyl protons at δ 1.69 (3H, s) to the neighboring carbons at C-6, -7, and -8 (δ 47.9, 141.2, 124.6). And also HMBC correlations from

H-2, -3 and -4 to the carbonyl carbon at C-20 (δ 178.8) provided the attachment of the carbonyl group at the C-3 position of the cyclohexane. Next, the remaining molecular formula, C₂H₅NO₃Na, was determined to be constituted by two methylenes, an amine and three heteroatoms (Na, S, O) based on the ¹³C-NMR chemical shift values and the COSY spectrum. An ethylene unit was readily assigned by two triplet proton signals at δ 3.57 and 2.94 which were not accommodated in diterpene, and linked with the given diterpenoid through an amide bond on the basis of the HMBC correlation of the methylene protons at δ 3.57 (2H, t, *J* = 6.8 Hz) with the carbonyl carbon at C-20 (δ 178.8). An amide moiety was supported by the typical absorption band at 1637 and 1453 cm⁻¹ in the IR spectrum and the chemical shift value of C-2' (δ 51.4) suggested a sulfur-bearing carbon, not an oxygen-bearing one. Thus, the molecular formula, C₂H₅NO₃Na, was elucidated to be a taurine residue.⁸⁾

The geometry of the double bonds for 1 was determined by the large coupling constants, *J* = 15.1 Hz for the olefinic protons at C-9, -10 and the NOE correlations on H-9/H-18, H-9/H-19, H-8/H-10, and H-14/H-16, indicating that all of the double bonds had the (*E*) configurations. And 1,4-symmetrical cyclohexane was formed to be the chair form from the NOE correlations on H-3/H1β, H-3/H5β and H-6/H2α, H-6/H4α. In addition, to determine the configuration at C-11, a chemical degradation was performed.⁹⁾ Treatment of 1 with NaIO₄ in the presence of RuCl₃ as a catalyst gave (*S*)-2-methylglutaric acid, which was confirmed by both comparison of the ¹H-NMR spectrum with an authentic sample and measurement of optical rotation {[α]_D²⁴ + 23° (*c* = 0.02, MeOH)}.¹⁰⁾ Therefore, the molecular structure of 1 was determined to be sodium 1'-[(6-[(7*E*, 9*E*)-7,11,15-trimethyldeca-7,9,14-trien-7-yl]cyclohexyl)carbonyl]amino]-

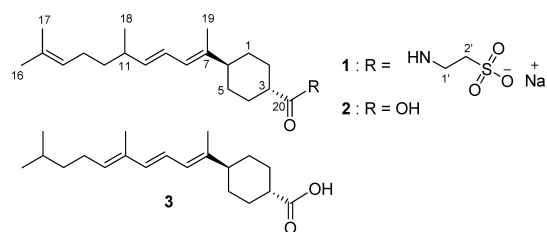


Fig. 1. Chemical Structures of Phorbasins G–I (1–3) Isolated from the Sponge *Phorbas gukulensis*

Table 1. NMR Spectral Data of Phorbasins G (**1**) and I (**3**) in CD₃OD

Position	Phorbasin G (1)			Phorbasin I (3)	
	δ_C , mult.	δ_H (J in Hz)	HMBC	δ_C , mult.	δ_H (J in Hz)
1, 5	32.0, CH ₂	1.76, br dd (12.5, 2.8), 1.31, m	2, 4, 6	31.9, CH ₂	1.77, br dd (12.9, 3.2) 1.32, qd (12.9, 3.2)
2, 4	30.7, CH ₂	1.89, br d (12.5) 1.48, dd (12.5, 3.4)	1, 3, 5 1, 3, 5, 20	30.5, CH ₂	2.03, br dd (12.9, 3.2) 1.45, qd (12.9, 3.2)
3	46.3, CH	2.12, tt (12.5, 3.4)	2, 4, 20	44.5, CH	2.22, tt, (12.2, 3.7)
6	47.9, CH	1.89, m	1, 5	48.2, CH	1.92, tt, (11.9, 10.8)
7	141.2, qC			142.6, qC	
8	124.6, CH	5.78, d (10.8)	6, 9, 10, 19	125.3, CH	5.88, d (10.8)
9	126.5, CH	6.20, dd (15.1, 10.8)	7, 8, 11	123.8, CH	6.34, dd (15.1, 10.8)
10	139.4, CH	5.39, dd (15.1, 8.3)	9, 11, 18	137.2, CH	6.13, d (15.1)
11	38.0, CH	2.14, m	9, 10, 12, 18	135.3, qC	
12	38.5, CH ₂	1.31, m	11, 14	133.3, CH	5.43, t (7.3)
13	26.9, CH ₂	1.94, dd (14.9, 7.1)	12, 14, 15	27.2, CH ₂	2.15, dt (7.8, 7.3)
14	125.8, CH	5.08, tq (7.1, 1.2)	13, 17	40.0, CH ₂	1.26, dt (8.8, 6.8)
15	132.1, qC			28.9, CH	1.55, quint (6.6)
16	25.9, CH ₃	1.66, br s	14, 15, 17	22.9, CH ₃	0.88, d (6.6)
17	17.8, CH ₃	1.57, br s	14, 15, 16	22.9, CH ₃	0.88, d (6.6)
18	21.4, CH ₃	0.98, d (6.8)	10, 11, 12	12.5, CH ₃	1.76, s
19	15.0, CH ₃	1.69, s	6, 7, 8	15.1, CH ₃	1.75, d
20	178.8, qC			180.2, qC	
1'	36.5, CH ₂	3.57, t (6.8)	20, 2'		
2'	51.4, CH ₂	2.94, t (6.8)	1'		

ethanesulfonate.

Phorbasin H (**2**) was isolated in a yellowish gum and established as a molecular formula C₂₀H₃₂O₂ on the basis of high resolution FAB-MS and ¹³C-NMR data. This molecule contained five double bond equivalents and was made up of four methyls, eight methylenes, seven methines, and three quaternary carbons as analyzed by a combination of the ¹H-NMR and the edited HSQC spectra. The ¹H- and ¹³C-NMR spectra of **2** were highly similar to those of **1**, except for the absence of two methylene signals and a difference in the carbon chemical shift in the carbonyl group. The intense absorption band at 1704 cm⁻¹ in the IR spectrum and the molecular formula suggested the presence of the carboxyl group. Thus, the structure of **2** produced from the loss of a taurine moiety could be deduced to be the basic skeleton of **1**. Accordingly, **2** was determined to be 6-[(7*E*,9*E*)-7,11,15-trimethyldeca-7,9,15-trien-7-yl]cyclohexanecarboxylic acid.

Phorbasin I (**3**) was obtained as a yellow gum and its molecular formula was given to be the same as that of **2** by the high resolution FAB-MS. However, the UV and NMR spectra of **3** were different from those of **1** and **2**. The ¹H-NMR spectrum of **3** showed a characteristic large methyl peak corresponding to a dimethyl group, and two broad singlet methyl signals. The sequential couplings from the dimethyl protons at δ 0.88 to the olefinic proton at δ 5.43 were observed in the COSY and TOCSY spectra. From the interpretation of the 2D NMR spectra, the only structural difference from **2** was the position of one double bond placed between C-11 and -12. Stereochemical assignment of the $\Delta^{11,12}$ double bond was established as an (*E*) configuration based on the NOE correlation on H-10/H-12. Therefore, **3** was determined to be 6-[(7*E*,9*E*,11*E*)-7,11,15-trimethyldeca-7,9,11-trien-7-yl]cyclohexanecarboxylic acid.

In the course of finding an activator for the adenosine monophosphate kinase (AMPK) protein in cells from marine specimens,^{11,12)} we isolated three new phorbasin compounds.

Unlike the previous phorbasins A–F, phorbasins G–I have a symmetrical cyclohexane moiety and a carbonyl group at the C-20 position. In particular, phorbasin G (**1**) was determined as a diterpenoid containing a taurine residue. Very recently, three other diterpenyl-aurines, containing phorbasins D similar to phorbasin G, were reported from an Australian marine sponge, *Phorbas* sp.¹³⁾

Unfortunately, phorbasins G–I did not exhibit a significant activation effect on AMPK, though phorbasins H and I showed a weak effect to a concentration of 10 μ M in a Western blot analysis. We are under collaboration for investigation into other biological activities.

Experimental

General Optical rotations were measured on a JASCO P-1010 polarimeter with a 5 cm cell. UV spectra were obtained in MeOH using a Shimadzu UV-1700 and IR spectra were measured on a JASCO FT/IR 4100 spectrometer. All NMR spectra were recorded in a CD₃OD solution on a Varian VNMRS 500 spectrometer. Chemical shifts of the proton and carbon spectra were reported in reference to residual solvent peaks at 3.30 ppm and 49.0 ppm, respectively. High resolution mass spectra were obtained on a JEOL JMS-700 spectrometer and mass spectrometric analysis was performed using a four-sector tandem mass spectrometer (JMS-HX110/110A, JEOL) with the mass resolution set at 1000 (10% valley).

Animal Material The specimens of *Phorbas gukulensis* (Sample No. 07G-6) were collected by hand using SCUBA at a depth of 30 m in 2007 off the shore of Gagu-Do at the West Sea, Korea. The sponge had a thick mass; it measured 110×95 mm and was 20 mm thick. Oscules were very rare and the texture was very soft. The color in life was red. In the skeleton, the megascleres were tornotes (340–420×6–10 μ m), small acanthostyles (160–195×5–10 μ m), and the large acanthostyles (390–470×8–11 μ m), and microscleres were isochelas (25–30 μ m). A voucher specimen is deposited at the Natural History Museum, Hannam University, Korea.

Extraction and Isolation The frozen organism (1.2 kg) was cut into small pieces and extracted twice with MeOH at room temperature. The methanolic extract (ca. 140 g) was partitioned between the *n*-BuOH and H₂O layers. The butanolic layer was evaporated under reduced pressure and repartitioned between *n*-hexane and 15% aqueous MeOH for defatting. Then the aqueous MeOH fraction was subjected to reversed-phase silica gel flash column chromatography eluting with solvents of decreasing polarity (MeOH/H₂O=50/50, 60/40, 70/30, 80/20, 90/10; 100% MeOH; acetone) to

give seven fractions. Compound **1** was isolated from a 30% aqueous MeOH fraction by reversed phase HPLC (YMC ODS-A column, 250 mm×10 mm ID) eluting with 35% aqueous MeOH to yield 5.5 mg. Compounds **2** and **3** were obtained from a 100% MeOH fraction after purification on reversed-phase HPLC using 10% aqueous MeOH to obtain 5.0 mg and 2.6 mg, respectively.

Phorbasin G (**1**) was obtained as a pale yellowish gum. ^1H - and ^{13}C -NMR data are given in Table 1. $[\alpha]_{\text{D}}^{24} + 30.1^\circ$ ($c=0.10$, MeOH). UV λ_{max} (MeOH) nm (log ϵ): 242 (4.12). IR (film) cm^{-1} : 2930, 2865, 1637, 1453, 1210, 1039. HR-FAB-MS m/z : 456.2159 (Calcd for $\text{C}_{22}\text{H}_{36}\text{NO}_4\text{SNa}_2$: 456.2157). LR-FAB-MS/MS m/z : 456.1, 372, 386, 224, 140, 126.

Phorbasin H (**2**) was obtained as a pale yellowish gum. ^1H -NMR (CD_3OD), δ : 6.21 (1H, dd, $J=15.1$, 11.4 Hz, H-9), 5.80 (1H, br d, $J=11.4$ Hz, H-8), 5.40 (1H, dd, $J=15.1$, 8.3 Hz, H-10), 5.09 (1H, tq, $J=7.3$, 1.4 Hz, H-14), 2.22 (1H, tt, $J=12.4$, 3.7 Hz, H-3), 2.14 (1H, m, H-11), 2.03 (2H, dd, $J=12.4$, 3.2 Hz, H-2 β , 4 β), 1.95 (2H, quint, $J=7.3$ Hz, H-13), 1.89 (1H, tt, $J=12.4$, 2.8 Hz, H-6), 1.78 (2H, dd, $J=12.4$, 2.8 Hz, H-1 α , 5 α), 1.71 (3H, d, $J=0.9$ Hz, H-19), 1.67 (3H, s, H-16), 1.59 (3H, s, H-17), 1.46 (2H, qd, $J=12.4$, 3.7 Hz, H-2 α , 4 α), 1.34 (2H, qd, $J=12.4$, 3.2 Hz, H-1 β , 5 β), 1.31 (2H, m, H-12), 0.98 (3H, d, $J=6.9$ Hz, H-18); ^{13}C -NMR (CD_3OD) δ : 180.2 (C, C-20), 141.2 (C, C-7), 139.4 (CH, C-10), 132.1 (C, C-15), 126.5 (CH, C-9), 125.8 (CH, C-14), 124.7 (CH, C-8), 48.0 (CH, C-6), 44.5 (CH, C-3), 38.5 (CH_2 , C-12), 38.0 (CH, C-11), 31.9 (CH_2 , C-1, -5), 30.5 (CH_2 , C-2, -4), 26.9 (CH_2 , C-13), 25.9 (CH_3 , C-16), 21.4 (CH_3 , C-18), 17.8 (CH_3 , C-17), 15.0 (CH_3 , C-19). $[\alpha]_{\text{D}}^{24} + 51.2^\circ$ ($c=0.10$, MeOH). UV λ_{max} (MeOH) nm (log ϵ): 242 (4.31). IR (film) cm^{-1} : 2922, 2858, 1704, 1450. HR-FAB-MS m/z : 327.2304 (Calcd for $\text{C}_{20}\text{H}_{32}\text{O}_2\text{Na}$: 327.2300).

Phorbasin I (**3**) was obtained as a pale yellowish gum. ^1H - and ^{13}C -NMR data are given in Table 1. UV λ_{max} (MeOH) nm (log ϵ): 244 (3.35), 278 (3.71). IR (film) cm^{-1} : 3348, 2940, 1708, 1028. HR-FAB-MS m/z : 305.2481 (Calcd for $\text{C}_{20}\text{H}_{33}\text{O}_2$: 305.2481).

Oxidative Cleavage of Phorbasin G To a biphasic solution of 5.4 mg (0.012 mmol) of phorbasin G and 21.7 mg (0.101 mmol) of NaIO_4 in a mixture of 1 ml of CCl_4 , 1 ml of CH_3CN , and 1.5 ml of H_2O was added 15.2 mg of $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$. After vigorous stirring the mixture for 2 h at room tempera-

ture, the solvents were removed under vacuum. The residue was redissolved in MeOH and filtered. The filtrate was dried and separated by reversed-phase HPLC (YMC ODS column, 5% aqueous MeOH) to give 0.8 mg of 2-methylglutaric acid: $[\alpha]_{\text{D}} + 23^\circ$ ($c=0.02$, MeOH); ^1H -NMR (CDCl_3) δ : 2.45 (1H, m), 2.32 (2H, m), 1.89 (1H, m), 1.72 (1H, m), 1.16 (3H, d, $J=6.8$ Hz).

Acknowledgments This research was supported by a grant from Marine Biotechnology Project funded by Ministry of Land, Transport and Maritime Affairs, and the Brain Korea 21 (BK21) project, Ministry of Education & Human Resources Development, Republic of Korea.

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