ORGANIC LETTERS

2009
Vol. 11, No. 24
5590−5593

Phorbaketals A, B, and C, Sesterterpenoids with a Spiroketal of Hydrobenzopyran Moiety Isolated from the Marine Sponge *Phorbas* sp.

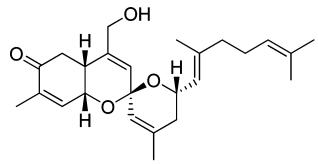
Jung-Rae Rho,*,† Buyng Su Hwang,† Chung Ja Sim,‡ Seewon Joung,§ Hee-Yoon Lee,§ and Hyeon-Jin Kim||

Department of Oceanography, Kunsan National University, Jeonbuk, 573-701, South Korea, Department of Biological Sciences, College of Life Science and Nano Technology, Hannam University, Daejeon, 305-811, South Korea, Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon, 305-701, South Korea, and Jinis BDRD Institute, Dunsan, Bongdong, Jeonbuk, 565-902, South Korea

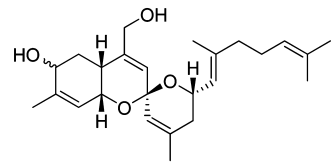
jrrho@kunsan.ac.kr

Received September 25, 2009

ABSTRACT



Phorbaketal A



(*R*)-OH Phorbaketal B
(*S*)-OH Phorbaketal C

Three new sesterterpenoids, phorbaketals A (1), B (2), and C (3) which have a spiroketal of the hydrobenzopyran moiety, were isolated from the Korean marine sponge *Phorbas* sp. Their complete structures were elucidated by spectral and chemical methods. They exhibited moderate cytotoxicity against human colorectal, hepatoma, and lung cancer cell lines. Furthermore, the cultivation of the bacterial fraction from the sponge afforded compound 1.

Marine sponges have been reported as a major source of a structurally novel class of secondary metabolites with diverse biological activities.[1] Of marine sponges, the genus *Phorbas* has been known to produce various potent bioactive compounds with unique structures since the first isolation of phorbazoles in 1994.[2] Our search for bioactive compounds from this specimen yielded cytotoxic gagunins and new phorbasin derivatives.[3]

During our continuing search for new natural products through bioactivity guided screening from Korean species,

† Kunsan National University.
‡ Hannam University.
§ Korea Advanced Institute of Science and Technology.
|| Jinis BDRD Institute.

(1) (a) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2009**, *26*, 170–244, and earlier reports in the series. (b) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 153–156. (c) Leone, P. A.; Carroll, A. R.; Towerzey, L.; King, G.; McArdle, B. M.; Kern, G.; Fisher, S.; Hooper, J. N. A.; Quinn, R. J. *Org. Lett.* **2008**, *10*, 2585–2588.

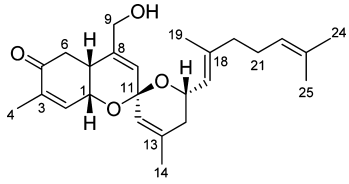
(2) (a) Rudi, A.; Stein, Z.; Green, S.; Goldberg, I.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *Tetrahedron Lett.* **1994**, *35*, 2589–2592. (b) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126–8131. (c) Vuong, D.; Capon, R. J. *J. Nat. Prod.* **2000**, *63*, 1684–1685. (d) McNally, M.; Capon, R. J. *J. Nat. Prod.* **2001**, *64*, 645–647. (e) Jang, K. H.; Jeon, J.-E.; Ryu, S.; Lee, H.-S.; Oh, K.-B.; Shin, J. *J. Nat. Prod.* **2008**, *17*, 1701–1707. (f) Skepper, C. K.; MacMillan, J. B.; Zhou, G. X.; Masuno, N. N.; Molinski, T. F. *J. Am. Chem. Soc.* **2007**, *129*, 4150–5150. (g) Morinaka, B. I.; Masuno, M. N.; Pawlik, J. R.; Molinski, T. F. *Org. Lett.* **2007**, *9*, 5219–5222. (h) Zhang, H.; Capon, R. J. *Org. Lett.* **2008**, *10*, 1959–1962.

(3) (a) Shin, J.; Rho, J.-R.; Lee, H.-S.; Sim, C. J. *Tetrahedron* **2002**, *58*, 9585–9591. (b) Lee, H.-S.; Park, S. Y.; Sim, C. J.; Rho, J.-R. *Chem. Pharm. Bull.* **2008**, *56*, 1198–1200.

10.1021/ol902223m
Published on Web 11/18/2009

recently we encountered novel tricyclic sesterterpenoids from other *Phorbas* sp. and named them phobaketals that possess an unprecedented novel spiro[5.5]ketal fused to a hydrobenzopyran ring. Among more than ten derivatives of the phorbaketal that were purified from the specimen, phorbaketal A (**1**) was isolated in an unusually large amount, while other derivatives were isolated in small quantities. Contrary to many occasions in marine natural products research that suffered from limited supplies of the compounds for comprehensive biological and pharmacological evaluation,[4] this relative abundance of phorbaketal A (**1**) would allow us a chance to evaluate diverse biological activities and further pharmacological studies. Herein we report the isolation and structural elucidation of three new major compounds and their biological activities as they exhibited moderate cytotoxic activity against human colorectal, hepatoma, and lung cancer cell lines.

A specimen of *Phorbas* sp. (dry weight 500 g) was collected by hand using SCUBA off the shore of Gageo Island, South Korea, in 2007. The sponge was extracted twice with MeOH. The combined extract was partitioned between CH_2Cl_2 and H_2O, and then the organic layer was further repartitioned between 15% aqueous MeOH and *n*-hexane. The polar layer (ca. 5 g) was fractionated by reversed-phase silica flash column chromatography, followed by reversed-phase silica HPLC to afford phorbaketal A (**1**, 1.2 g, 0.24%), B (**2**, 30 mg, 0.006%), and C (**3**, 80 mg, 0.016%) (for details, see the Supporting Information).



Phorbaketal A (**1**)

Phorbaketal A (**1**) was isolated as a yellowish oil and determined to have a molecular formula of $C_{25}H_{34}O_4$ on the basis of high-resolution FABMS ($[M + H]^+$ peak at m/z = 399.2535), consistent with nine degrees of unsaturation. The IR spectrum revealed the strong absorption bands at 3434 (hydroxyl), 1681 (carbonyl), and 1446 cm^{-1} (double bond). The ^{13}C NMR spectrum of **1** displayed ten olefinic resonances (δ_c 123.0, 124.7, 125.0, 125.8, 132.6, 138.7, 139.5, 141.6, 142.0, 143.7), a ketone (δ_c 202.7), and a characteristic quaternary signal (δ_c 96.1) typical of a doubly oxygenated carbon (Table 1). This information led to the conclusion that the remaining unsaturations were accounted for by the presence of three rings in compound **1**. Besides, five vinyl methyls, five methylenes, and four methine carbons in the aliphatic region were identified from the ^1H and edited HSQC spectra.

Interpretation of the $^1H-^1H$ COSY and HSQC spectra led to three substructures, **i**, **ii**, and **iii** (the bold lines in Figure 1), along with the assignment of protons and the corresponding carbons. The identity of ring A was established by the HMBC correlations between a vinyl methyl proton and two

Table 1. ^1H and ^{13}C NMR Data of Phorbaketal A (**1**)[a]

no.	δ_C	δ_H (mult, *J* Hz)
1	64.7, d	4.49, dd (5.5, 3.3)
2	141.6, d	6.68, dd (5.5, 1.2)
3	139.5, s	
4	15.9, q	1.81, s
5	202.7, s	
6a	38.8, t	2.43, dd (16.1, 13.7)
6b		2.58, dd (16.1, 3.9)
7	34.7, d	2.59, ddd (13.7, 3.9, 3.4)
8	143.7, s	
9a	63.8, t	4.03, d (14.7)
9b		4.07, d (14.7)
10	124.7, d	5.54, br s
11	96.1, s	
12	123.0, d	5.28, br s
13	138.7, s	
14	22.8, q	1.75, s
15a	36.4, d	1.85, dd (17.6, 3.4)
15b		2.02, dd (17.6, 11.3)
16	66.9, d	4.74, ddd (11.3, 8.1, 3.4)
17	125.8, d	5.22, br d (8.1)
18	142.0, s	
19	16.9, q	1.77, s
20	40.6, t	2.05, m
21	27.5, t	2.13, m
22	125.0, d	5.12, br t (7.4)
23	132.6, s	
24	25.9, q	1.68, s
25	17.8, q	1.61, s

[a] Measured at 500 MHz (^1H) and 125 MHz (^{13}C) in CD_3OD.

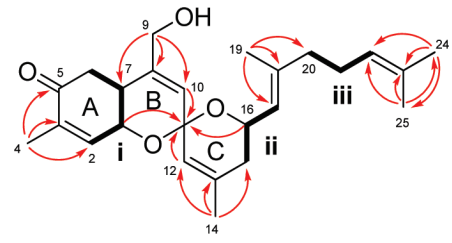


Figure 1. Key HMBC correlations (H→ C, red lines) of **1**.

olefinic carbons and a ketone group which was in turn correlated by two methylene protons at C-6. Furthermore, each of the remaining four vinyl methyl protons was commonly correlated with two olefinic carbons and one aliphatic carbon in the HMBC spectrum, explaining the presence of three trisubstituted double bonds. These observations readily allowed us to connect three double bonds via substructures **ii** and **iii** in linear fashion. Both the olefinic proton at the end of the chain and the unassigned olefinic proton at δ_H 5.54 correlated to the doubly oxygenated quaternary carbon at δ_C 96.1 in the HMBC spectrum.

(4) Molinski, T. F.; Dalisay, S.; Lievens, S. L.; Seludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69–85.

Moreover, this carbon, on the basis of HMBC correlations, connected simultaneously with C-1 and C-16 through ether bonds, forming a ketal group.

Finally, an AB splitting oxymethylene proton showed a coupling with the proton at δ_H 5.54 in the COSY spectrum as well as correlation to two olefinic carbons and C-7 of the ring A in the HMBC spectrum. This correlation led to the conclusion that ring B fused to the ring A is again connected with the ring C by a spiro junction, forming a spiro[5.5]ketal ring.

The relative configuration on the chiral centers of **1** was assigned by ROESY analysis and the values of coupling constant (Figure 2). The methine proton H-1 exhibited strong

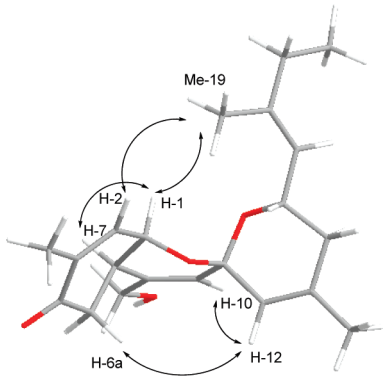


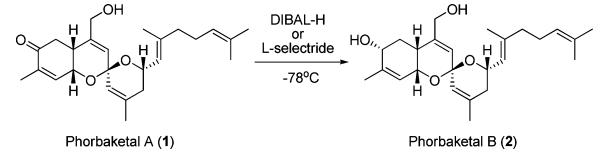
Figure 2. Key NOE correlations of **1**.

NOE correlation with H-7, indicating that the A/B ring junction has a *cis* configuration. Furthermore, the configuration of the spiro junction of the ring B and the ring C was evident from the NOE correlation between the olefinic proton H-10 in the ring B and the other olefinic proton H-12 in the ring C which also showed a weak ROESY signal with the proton H-6a. Similarly, the NOE correlations of Me-19/H-1, Me-19/H2, and Me-19/H-16 provided a clue for the placement of the side chain on the ring C. The large coupling constant of the proton H-15b [δ_H 2.02 (dd, 17.6, 11.3)] indicated the *anti* relationship with H-16, and the strong NOE correlation of H-17 with H-15b confirmed the configuration at C-16.

Phorbaketal B (**2**) was determined as $C_{25}H_{36}O_4$ on the basis of a combination of HRFABMS (*m/z* 401.2692 measured for $[M + H]^+$) and the ^{13}C spectrum. Compared with **1**, the obvious difference is the absence of a carbonyl group from the lack of a characteristic signal in the IR and ^{13}C spectra. On the basis of the molecular formula, compound **2** is supposed to possess a new hydroxyl group instead of carbonyl group of the compound **1**. This was confirmed by an HMBC correlation of the vinyl methyl Me-4 with a new carbon bearing a hydroxyl moiety that replaced the ketone group. This prompted us to decipher the conversion of **1** to **2** through a reduction process at the C-5 position in the ring A. Except for this modification, the other structural feature of compound **2** was identical to that of **1** in the comparison of chemical shifts and analysis of 2D NMR data. Even the relative stereochemistry was consistent. However, the stereochemistry of a newly formed chiral center at C-5 had to be determined by the coupling constant value, together with the ROESY data. All of the large coupling constants for H-6a [δ_H 1.51 (ddd, 12.5, 11.5, 10.3)] at the most upfield region mean that the two adjacent protons are located in the *anti* position to each other. On the basis of this consideration, both H-5 and H-7 should be placed at the *anti* position with H-6a in the ring A. Accordingly, the proton H-5 was oriented in the same direction as the protons H-7. This was supported by definite NOE correlations of H-7 and H-5.

Stereochemistry of the C-5 hydroxyl group and the structural relationship between the compounds **1** and **2** was also confirmed by diisobutylaluminium hydride reduction of **1** that produced the compound **2** stereoselectively. Reduction of the compound **1** using L-selectride with an anticipation of the formation of the diastereomeric product of compound **2**[5] also produced the compound **2** stereoselectively. These results also support the structural integrity of the compound **1** as it would allow only the β-face of the ketone for any hydride addition reaction (Scheme 1).

Scheme 1. Conversion of Phorbaketal A (**1**) into Phorbaketal B (**2**)



The absolute stereochemistry of compounds **1** and **2** could be established by the modified Mosher's method[6] for **2**. Esterification of **2** with *R*-(−)- and *S*-(+)-MTPA chlorides led to an *S*-MTPA ester of **2** and an *R*-MTPA ester of **2**, respectively. All proton signals in the two ester derivatives were assigned and the differences in chemical shifts between the corresponding protons in the ^1H NMR spectra of the *S*/*R*-MTPA esters were calculated (Figure 3). This concluded that the absolute configuration of C-5 was *R*.

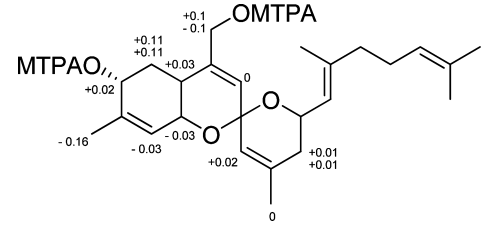


Figure 3. ^1H NMR chemical shift differences ($\Delta\delta^{S-R}$) in ppm for *S*-/*R*-MTPA esters of **2** in $CDCl_3$.

Phorbaketal C (**3**) had the same molecular formula $C_{25}H_{36}O_4$ as **2**, on the basis of HRFABMS data (*m/z*

401.2692 for $[M + H]^+$, Δ −0.4 mmu), indicating eight degrees of unsaturation. The chemical shift values in the ^1H and ^{13}C spectra of **2** and **3** are very similar within an error of ~0.1 ppm, respectively, except for those in the position of C-3 ~ C-7. The 2D NMR data revealed the planar structure of **3** to be identical to **2**. Thus, it is inferred that the difference in chemical shifts came from the different stereochemistries on C-5 and/or C-7. A crucial clue came from the fact that the proton coupling constant at H-6a of **3** was observed as J_{HH} = 13.2, 13.2, 3.7 Hz, indicating one of the above chiral centers to the *anti* direction relative to H-6a. From the measurement of coupling constant of H-7, it is concluded that the position of the hydroxyl moiety at C-5 was opposite to that of **2**.

To the best of our knowledge, the tricyclic skeleton of phorbaketals A (**1**), B (**2**), and C (**3**) is a new frame, and a spiroketal of hydrobenzopyran moiety is unprecedented in natural products. Compounds **2** and **3** are isomers presumed to be originated from **1** during the reduction process by the sponge. Interestingly, the amount of phorbaketal A (**1**) from our specimen was relatively very large, compared with those of **2** and **3**. This phenomenon led us to speculate that the original source of phorbaketal A (**1**) might stem from symbionts habitating in the sponge. To preliminarily test this hypothesis, the mixed microbial cultures obtained from the homogenized sponge were further cultivated in the YM liquid medium at 20 °C. As we anticipated, phorbaketal A (**1**) was identified from the extract of the cultivated cells with ca. 4 mg of purified compound from the 100 mL culture. Presently, the isolation of the corresponding pure strain which produces the compound is in progress. On the basis of our preliminary test, we believe that phorbaketal A (**1**) was originated from an endosymbiotic microorganism in the sponge.

The cytotoxicity test for phorbaketals A, B, and C was carried out against three human cancer cell lines using the colorimetric methylthiazole tetrazolium bromide (MTT) assay. Phorbaketal A (**1**) exhibited mild activities against human colorectal cancer HT-29 with an IC_{50} value of 12 μg/mL, hepatoma cancer HepG2 with an IC_{50} value of 11.2 μg/mL, and lung cancer A549 with an IC_{50} value of 11 μg/mL. Phorbaketals B (**2**) and C (**3**) showed the activities given as follows: human HT-29 cancer cell (IC_{50} 27.9 and 212 μg/mL), HepG2 cell (IC_{50} 14.8 and 11.8 μg/mL), and A549 cell (IC_{50} 465 and 12.4 μg/mL), respectively.

Acknowledgment. We appreciate professor S. Matsunaga at University of Tokyo, Japan, and Professor W. Yih at Kunsan National University for kind comments and constructive discussion. This research was supported by the Marine Biotechnology Program funded by the Ministry of Land, Transport and Maritime Affairs of Korea. B. S. Hwang thanks the support by the Brain Korea 21 (BK21) Project, Ministry of Education & Human Resources Development, Republic of Korea.

Supporting Information Available: Experimental procedures and spectral data of **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL902223M

(5) (a) Dhulut, S.; Bourin, A.; Lannou, M.-I.; Fleury, E.; Lensen, N.; Chelain, E.; Pancrazi, A.; Ardisson, J.; Fahy, J. *Eur. J. Org. Chem.* **2007**, 5235–5243. (b) Ceccarelli, S. M.; Piarulli, U.; Gennari, C. *Tetrahedron* **2001**, *57*, 8531–8542.

(6) (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096. (b) Tsuda, M.; Toriyabe, Y.; Endo, T.; Kobayashi, J. *Chem. Pharm. Bull.* **2003**, *51*, 448–451.