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Phorone A and Isophorbasone A, Sesterterpenoids Isolated from the Marine Sponge *Phorbas* sp.

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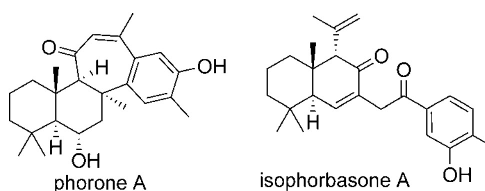
ABSTRACT



A chemical investigation of a Korean marine sponge, *Phorbas* sp., yielded unprecedented sesterterpenoids phorone A (1) and isophorbasone A (2) along with ansellone B (3) and phorbasone A acetate (4). Their complete structures were elucidated by the combination of spectroscopic data and chemical manipulation. Phorone A (1) and isophorbasone A (2) have the new "phorane"(5) and "isophorbasane"(6) sesterterpenoid carbon skeletons, respectively. Ansellone B (3) and phorbasone A acetate (4) exhibited potent inhibitory activity on nitric oxide production in RAW 264.7 LPS-activated mouse macrophage cells with IC_{50} values of 4.5 and 2.8 μM, respectively.

Marine sponges of the genus *Phorbas* have been proven to be an abundant source of structurally novel secondary metabolites exhibiting diverse biological activities. Compounds that represent a variety of different classes have been reported including alkaloids,[1] macrolides,[2] diterpenoids,[3] steroids,[4] sesterterpenoids,[5] and tetraterpenoids[6] in the order of their discovery. These natural products displayed a wide range of biological activities such as antifungal activity,[2a] cytostatic activity,[2a] cytotoxicity,[2c,4a,5b,6,7] inhibition of isocitrate lyase,[7b]

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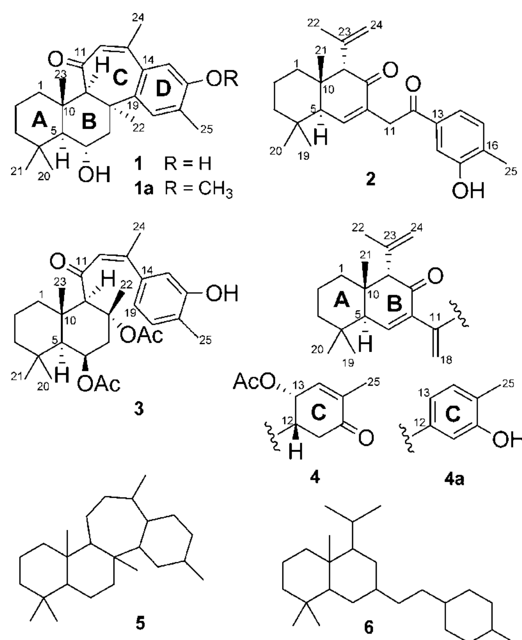
activation of the cAMP pathway,[5a,c] and induction of osteoblast differentiation.[5d]



During the course of our search for bioactive metabolites from Korean marine organisms, we encountered one species of sponge of the genus *Phorbas*. Chemical investigation of the extract uncovered a series of sesterterpenoids representing different carbon skeletons including phorone A (**1**), isophorbasone A (**2**), ansellone B (**3**), and phorbasone A acetate (**4**). Phorone A (**1**) and isophorbasone A (**2**) possess unprecedented tetracyclic "phorane" and tricyclic "isophorbasane" carbon skeletons, respectively. They are presumably biogenetically related to the previously reported phorbaketals, phorbasones, and ansellone. Details of the structural elucidation, plausible biogenic pathway, and biological activity of the compounds are presented below.

Phorone A (**1**) has a molecular formula of $C_{25}H_{34}O_3$ as established by high-resolution FABMS data of the $[M + H]^+$ ion at m/z 383.2583 (Δ −0.3 mmu). A combination the ^{13}C NMR data and edited HSQC data revealed the presence of six methyl groups, four methylenes, two methines, one oxymethine, three aliphatic quaternary carbons, one α,β-unsaturated ketone group, and six aromatic carbon resonances (Table 1). The aromatic ring and the α,β-unsaturated ketone group account for six of the nine sites of unsaturation required by the molecular formula and indicated that phorone A (**1**) contained three additional rings. The molecular formula of phorone A (**1**) contains three oxygen atoms according to the HRFABMS data. Therefore, the oxymethine carbon and the phenolic carbon must not be linked by an ether linkage but be substituted by free hydroxyl groups.

The major portion of the backbone of the tetracyclic terpene skeleton was established using COSY and HMBC correlations. COSY correlations permitted a linear connectivity of three contiguous diastereotopic methylenes in the range of 1.00−2.00 ppm and also a linear spin system starting with a doublet at δ 1.13 (H-5) continuing to a oxymethine at δ 4.03 (H-6) and ending with a diastereotopic methylene at δ 2.77 and 1.52 (H-7) (Figure 1). Each methyl singlet at δ 0.48 (H_3-23), 0.87 (H_3-21), 1.15 (H_3-20), and 1.17 (H_3-22) showed HMBC correlations to four neighboring carbons. These observations allowed the assignment of a partial structure of 3,4a,8,8-tetramethyldecahydronaphthalen-1-ol.

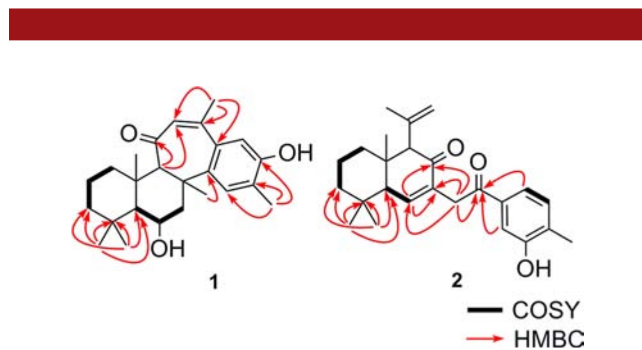


Figure 1. COSY and key HMBC correlations of **1** and **2**.

A tetrasubstituted phenyl ring was established by the carbon chemical shifts and the HMBC correlations. The methyl singlet at δ 2.28 (H_3-25) showed HMBC correlations to the aromatic carbons at δ 155.6 (C-16), 129.3 (C-17), and 130.5 (C-18). HMBC correlations from the aromatic resonance at δ 7.10 (H-15) to the aromatic carbons C-17 and C-19 (δ 138.5) and from the aromatic resonance at δ 7.39 (H-18) to the aromatic carbons C-14 (δ 136.0) and C-16 were observed. The methyl singlet at δ 2.35 (H_3-24) showed HMBC correlations to a pair of trisubstituted olefinic carbons at δ 132.3 (C-12) and 152.0 (C-13) and the aromatic carbon at δ 136.0 (C-14), demonstrating the connection of C-13 to the phenyl ring. HMBC correlation from H_3-22 to the aliphatic quaternary carbon C-8 and the aromatic carbon C-19 permitted the connection of C-8 to C-19. Connection between the α,β-unsaturated carbonyl carbon (C-11) and the aliphatic methine carbon (C-9) was secured by the observation of HMBC correlations from the methine proton resonance at δ 2.32 (H-9) to the carbonyl carbon resonance at δ 204.0 (C-11) and the olefinic carbon C-12, which revealed a seven-membered ring adjacent to the aromatic ring and decahydronaphthalene ring.

The relative configuration for **1** was determined by analysis of the coupling patterns and ROESY data. Owing to the overlap of H-1, 2, and 3 in the ^{1}H NMR spectrum, the coupling patterns were deduced using homonuclear decoupling experiments. Irradiation of the proton resonance at δ 1.52 (H-2a and H-7b) induced the change in coupling patterns of H-3b from a doublet of doublets of doublets (J = 13.4, 13.4, 4.1 Hz) to a doublet of doublets (J = 13.4, 4.1 Hz), which indicated that H-2a coupled to H-3b in 13.4 Hz and, accordingly, both H-2a and H-3b must be in the axial position. The appearance of H-7a changed from a doublet of doublets (J = 13.7, 3.1 Hz) to a doublet (J = 3.1 Hz); thus, one of the large coupling

Table 1. ^{1}H and ^{13}C NMR Data of Phorone A (**1**) in CD_3OD

no.	δ_H	mult (J in Hz)	δ_C	
1a	1.31	m	41.0	CH_2
1b	1.03	br d (13.9)		
2a	1.52	m	19.5	CH_2
2b	1.33	m		
3a	1.28	m	45.0	CH_2
3b	1.22	ddd (13.4, 13.4, 4.1)		
4			35.1	C
5	1.13	d (10.8)	63.7	CH
6	4.03	td (10.8, 3.1)	68.2	CH
7a	2.77	dd (13.7, 3.1)	53.4	CH_2
7b	1.52	m		
8			40.0	C
9	2.32	s	76.0	CH
10			41.7	C
11			204.0	C
12	6.30	s	132.3	CH
13			152.0	C
14			136.0	C
15	7.10	s	118.3	CH
16			155.6	C
17			129.3	C
18	7.39	s	130.5	CH
19			138.5	C
20	1.15	s	37.6	CH_3
21	0.87	s	22.4	CH_3
22	1.17	s	30.5	CH_3
23	0.48	s	18.5	CH_3
24	2.35	s	28.5	CH_3
25	2.28	s	16.6	CH_3

constants of H-6 (td, J = 10.8, 3.2 Hz) must be due to the coupling with H-7b, suggesting the axial orientation of H-7b. The strong 2D ROESY correlations between Me-23 and the three proton resonances of H-2a, H-6, and Me-21 revealed that the C-1 to C-10 decalin ring system was *trans* fused with Me-21, Me-23, and H-6 in the axial orientation. The same *trans*-fused decalin ring system was also observed for phorbasones,[5d] ansellone,[5c] and isophorbasone A (**2**), which is consistent with the proposal that they may share the same biosynthetic pathway. The 2D ROESY correlations between Me-22 and both H-7a and H-9 suggested that the rings B and C were *cis* fused.

In order to determine the absolute configuration of **1** by application of the modified Mosher's method, its 16-*O*-methyl derivative (**1a**) was first prepared by treatment with diazomethane. Esterification of **1a** with (R)-(−)- and (S)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride yielded (S)- and (R)-MTPA esters, respectively. The $\Delta\delta_{S-R}$ value distribution pattern suggested the S configuration at C-6 (Figure 2).

Isophorbasone A (**2**) gave a $[M + Na]^+$ ion at m/z 403.2244 in the HRFABMS, appropriate for a molecular formula of $C_{25}H_{32}O_3Na$. A detailed comparison of its NMR data with those of phorbasone A[5d] indicated that **2** contained the same ring system 5,5,8a-trimethyl-4a,5,6,7,8,8a-hexahydronaphthalen-2(1H)-one, which was substituted by an isopropene group as in phorbasone A. In addition to the

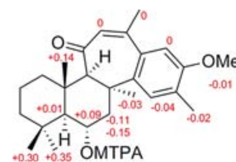


Figure 2. δ values ($\Delta\delta_{S-R}$) in ppm for (S)- and (R)-MTPA esters of 16-O-methyl ether of phorone A (**1a**) in CD_3OD.

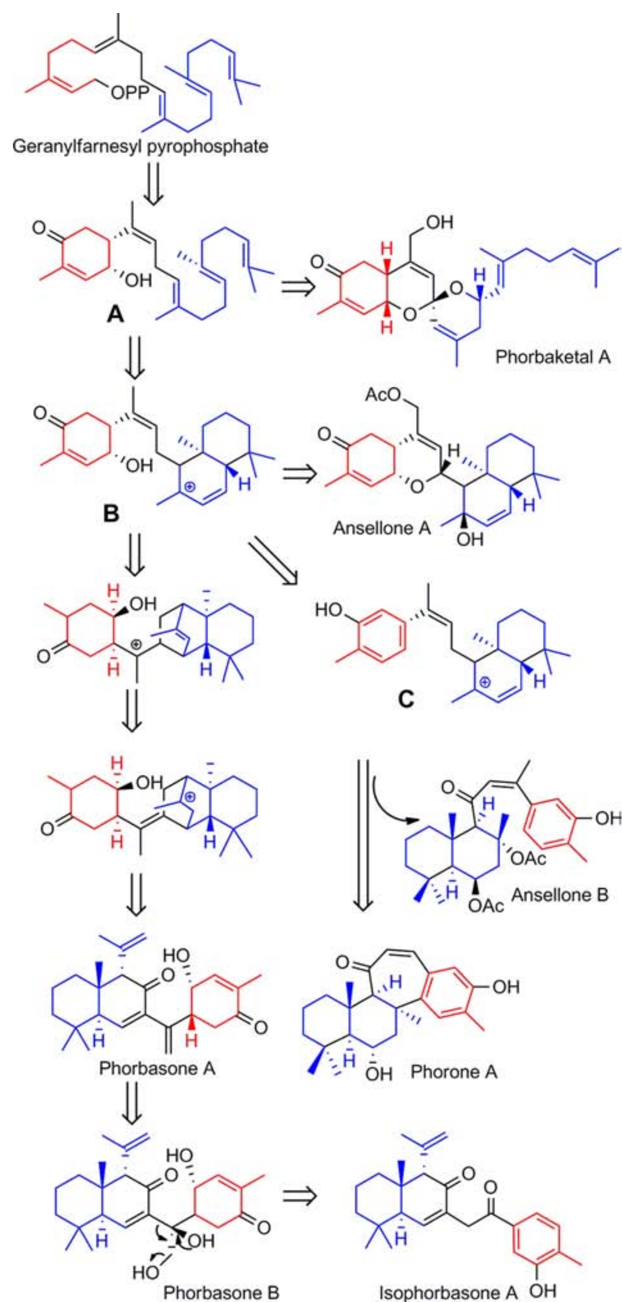


Figure 3. Proposed biosynthetic pathway to phorone and isophorbasone skeletons.

signals assigned to the bicyclic ring, an olefinic methyl carbon (δ 16.6), six aromatic carbons (δ 157.1, 137.4, 132.4, 131.9, 121.1, 114.7), a ketone carbonyl carbon (δ 199.7), and a methylene carbon (δ 40.4) were observed in the ^{13}C NMR spectrum. The former three groups of carbon signals and their HMBC correlations revealed the presence of a trisubstituted phenyl ring instead of the cyclohexenone moiety in phorbasone A and the attachment of C-12 (δ 199.7) on the phenyl ring. A pair of ^{1}H signals at δ 3.91 (H-11a) and 3.86 (H-11b) showed HMBC correlations to the carbons at δ 202.1 (C-8), 136.1 (C-7), and 151.9 (C-6) in the bicyclic unit and the benzoyl carbonyl carbon C-12, which assembled the two partial moieties of **2**. The relative configuration of **2** was assigned by ROESY analysis and the values of ^{1}H$-^{1}$H vicinal coupling constants. The large coupling constants between the protons H-1a (δ 1.47) and H-2a (δ 1.78) and between the protons H-2a and H-3b (δ 1.27) indicated that they were located in the axial position. The configuration of the bicyclic ring was established as *trans* ring fusion on the basis of the strong ROESY correlations among H-21 (δ 1.15), H-20 (δ 0.98), and H-2a and among H-5 (δ 2.61), H-1a, and H-3b. The ROESY correlations between H-21 and H-9 (δ 2.58) and between H-5 and H-24b (δ 4.83) indicated the *trans* relationship of Me-21 with the isopropene unit. The absolute configuration was established by comparison of the CD data with those of the ring C-aromatized derivative of phorbasone A (**4a**), which was produced by hydrolysis of **4** followed by aromatizaion with DBU (1,8-diazabicycloundec-7-ene). The CD spectrum of **2** revealed $\pi \rightarrow \pi^*$ Cotton effects (a positive Cotton effect around 202 nm and a negative Cotton effect around 236 nm) having the same sign as those of **4a**.

The surprising variety of sesterterpenoid skeletons isolated from this specimen has prompted us to report biosynthetic speculations in detail. The most realistic hypothesis suggests that all these sesterterpenoids derive from geranylfarnesyl pyrophosphate through cationic cyclizations and rearrangements. The early steps of the biosynthetic process were proposed to involve the initial cyclization of the geranyl unit and hydroxylation after removal of the pyrophosphate moiety to yield the intermediate **A** (phorbaketal carbon skeleton) (Figure 3). The farnesyl part of **A** undergoes cationic cyclization to give **B** (ansellone carbon skeleton), the last shared intermediate of ansellone, phorbasone, phorone, and isophorbasone. From a stereochemical point of view, this is a crucial step for formation of the *trans*-fused bicyclic structure shared by the four classes of sesterterpenoids. **B** first undergoes dehydroxylation and keto$-$enol tautomerization of its cyclohexenone ring before being subjected to further cyclization to yield the phorone carbon skeleton. The latter step could reasonably proceed via nucleophilic attack of the *para* carbon of the phenol ring on the C-8 carbocation. These proposals are supported by the key discovery of ansellone B. As an offshoot of the biosynthetic pathway, the stabilization of the C-8 carbocation through rearrangements as reported by Rho would generate the tricyclic ring of phorbasone A,[5d] which would be hydroxylated to give phorbasone B. The formation of isophorbasone A from phorbasone B could involve an oxidation of C-11, a dehydration and carbon shift of C-18 to C-7, and a dehydration and keto$-$enol tautomerization of the cyclohexenone ring to attain a more stable structure.

It was reported that effective suppression of NO production, via inhibition of inducible NOS (iNOS) expression, has become an alternative strategy for developing new compounds in the treatment of inflammatory disease.[8] The iNOS inhibitory assay was performed for compounds **1**$-$**4** by measuring NO production in RAW 264.7 LPS-induced mouse macrophage cells. Compounds **1** and **2** were not active up to 100 μM, while compounds **3** and **4** were found to exhibit potent inhibitory activity on nitric oxide production with IC_{50} values of 4.5 and 2.8 μM, respectively, which were better than those of prenylated flavonoids (IC_{50} values: 6.4$-$18.8 μM) from *Artocarpus communis*.[8b] In particular, compound **3** showed a favorable selectivity index (SI) of 3.8, which is indicative of its iNOS inhibitory activity without cytotoxicity.

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Supporting Information Available. Structural elucidation for compounds **3** and **4**, experimental section, spectroscopic data of **1a**, **1**$-$**4**, and **4a**, and biological activity data of compounds **1**$-$**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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