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A new derivative of phorbaketals isolated from a Marine Sponge *Phorbas* species

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Abstract : A new sesterterpenoid, phorbaketal derivative, was isolated from the marine sponge *Phorbas* species. Its planar structures was completely determined from a combination of extensive 1D and 2D NMR experiments and MS data, and also the stereochemistry on the chiral centers were established by the ROESY experiment and the comparison with the ^{1}H and ^{13}C chemical shifts of the known phorbaketal compounds. This compound **1** moderately showed cytotoxicity effect against hepatoma cancer HepG2 cell.

Keywords : 1D and 2D NMR, Phorbaketal, sponge *Phorbas* sp., cytotoxicity

INTRODUCTION

Marine sponges have had much attention as a new target for drug discovery because of the isolation of the structurally novel and biologically active compounds.[1-2] The marine sponge, *Phorbas* species, is a good example of this. This organism has been known to produce various potent bioactive compounds with unique structures since the first isolation of phorbazoles in 1994.[3-6] These structurally diverse natural products also showed a wide range of biological activities such as:

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antifungal activity, cytostatic acitivity, cytotoxicity, inhibition of isocitrate lyase, and activation of the cAMP pathway.[7-9]

Recently, we have reported novel skeletal sesterterpenoids phorbaketal and phorbasone from this organism collected in the Korean seawater.[10-11] These compounds exhibited mild activities against three human cancer cells and the effect on calcium deposition in the mesenthymal C3H10T1/2 cells, respectively. In the further isolation of these compounds for diverse screenings, we found a new derivative of phorbaketal A, in which has two acetyl groups. Here, we describe the isolation of compound **1** and its structure elucidation with NMR experiments.

EXPERIMENTAL

Extraction and Isolation

The marine sponge *Phorbas* sp. (07G-26) was collected from Gageo Island, South Korea and extracted twice with MeOH at room temperature. The methanolic extract was partitioned between CH_2Cl_2 and H_2O solvents and then the organic layer repartitioned between *n*-hexane and 15% aqueous MeOH for defatting. The MeOH fraction was performed on the vacuum column chromatography eluting with seven different solvent mixtures of MeOH and water. Among them, the fractions of 100% MeOH and 10% aqueous MeOH solvent had a large amount of phorbaketal compounds. Phorbaketal A, B, and C were mainly isolated from 10% aqueous MeOH fraction, while

compound **1**, diacetoxy phorbaketal, was obtained from 100% MeOH fraction. First, this fraction was partitioned into five subfraction (M1-M5) by using Sephadex LH20 open column chromatography. And then, M4 fraction (200mg) was separated by reversed phase HPLC(YMC ODS-A column, 150mm × 20mm, Varian RI detector) using a solvent system (H_2O / MeOH = 12 / 88) to yield compound **1**.

NMR experiment

The 1D and 2D NMR spectra were obtained on a Varian NMR system working at 500MHz for proton and 125MHz for carbon. The ^{1}H and ^{13}C NMR chemical shifts refer to CD_3OD at 3.30 and 49.0ppm, respectively. For all experiments, the temperature was stabilized at 297 K. The parameters used for 2D NMR spectra were as follows; a gradient COSY spectra were collected with a spectral width 2800 Hz in a 512 (t1) × 1024 (t2) matrix applying the pulse gradient of 1ms duration with a strength 10 G/m and processed with a sinebell function. The gradient HSQC and HMBC spectra were measured with J_{CH} = 140 Hz and $^{n}J_{CH}$ = 7Hz, respectively, and processed in a 256(t1) × 1024(t2) matrix by a linear prediction method for a higher resolution.

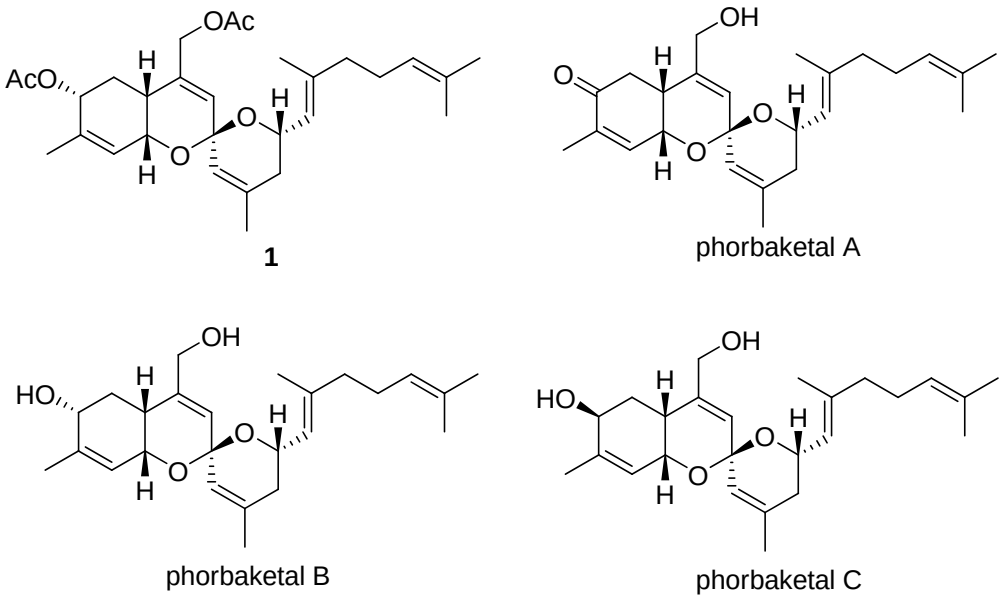


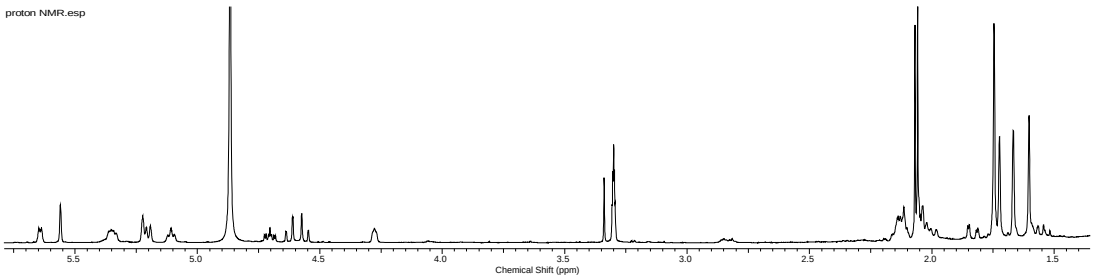
Fig 1. Four compounds isolated from the marine sponge *Phorbas* sp.

RESULTS AND DISCUSSION

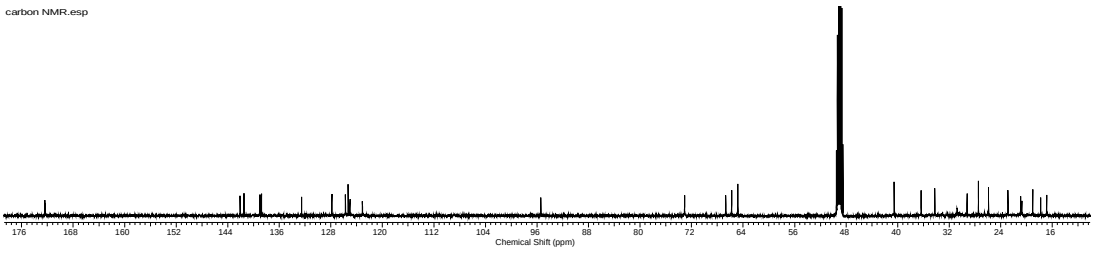
The 10% aqueous MeOH fraction of the extract was subjected on the LH20 open column chromatography to give five fractions (M1-M5). Among them, the M4 fraction was purified using ODS HPLC to afford compound **1**.

Compound **1** was isolated as a yellowish oil and its molecular formula was determined as $C_{29}H_{40}O_6$ on the basis of HRFABMS measurement ($[M+Na]^+$ ion peak m/z 507.2726, Δ=0.3), consistent with ten unsaturation degrees. IR spectrum showed a strong absorption band at 1237 cm^{-1}, indicating the presence of a carbonyl group and UV spectrum had an absorption band at 202.9 nm (ε 28765). This

compound had a specific optical rotation value, $[\alpha]^{25}_{D}$= -86.2(*c* = 0.15, MeOH). The ^{1}H and ^{13}C NMR spectra measured in CD_3OD were presented in Fig. 1. From the ^{13}C and DEPT experiments, **1** was revealed to have seven methyl, five methylene, nine methine and eight quaternary carbons. Especially, five singlet resonances in the range of 1.60 ~ 1.75 ppm are characteristic of the olefinic methyls and two methyls at 2.06 and 2.07 ppm indicated the presence of the acetyl groups together with the two carbonyl carbons at 172.3 and 172.4 ppm. Furthermore, the ten chemical shifts for sp2 carbons were indicative of the presence of five double bonds in **1**. This observation suggested that **1** possesses three ring units from the uncounted unsaturation degrees.



(a)



(b)

Fig 2. (a) Proton and (b) carbon NMR spectra of **1**.

Interpretation of the COSY, TOCSY and HSQC spectra provided three subunits: (I) CH(O)-CH_2-CH-CH(O)-CH=, (II) CH_2-CH(O)-CH=, (III) CH_2-CH_2-CH=. Two carbons at the both side of subunit (I) and the quaternary carbon at 141.5 ppm were correlated with the olefinic methyl proton at 1.72 ppm in the HMBC spectrum. This constructed a cyclohexene ring. In a similar way, the HMBC data also connected subunit (II) and (III) from the correlations between the olefinic methyl proton at 1.74 ppm and three carbons at 40.6, 125.0 and 142.1 ppm. On the other hands, **1** has a characteristic carbon chemical shift, 95.4 ppm, which could be assigned as a ketal carbon. This carbon was connected with two double bonds, which is determined by the strong HMBC correlations with two singlet olefinic protons at 5.22 and 5.56 ppm. Additional HMBC correlations as given in Fig. 3 (a) allowed us to assemble three fragments: the cyclohexene ring, the linear chain and the ketal moiety. The planar structure of **1** could be completed by two ether formations between the ketal carbon and two oxygen-bearing carbons at 64.8 and 66.7 ppm, respectively. This was also supported by the weak HMBC correlations between the ketal carbon and two protons at 4.28 and 4.70 ppm. Finally, the position of two acetyl groups was established by the HMBC correlations between their carbonyl carbons and the methine proton at 5.35 ppm and the methylene protons at 4.62, respectively. Accordingly, the structure of **1** was closely similar to that of phorbaketal B that was previously reported in this group, but a new derivative which has two acetyl functional groups in place of two hydroxyls at the C-6 and -1″ positions.

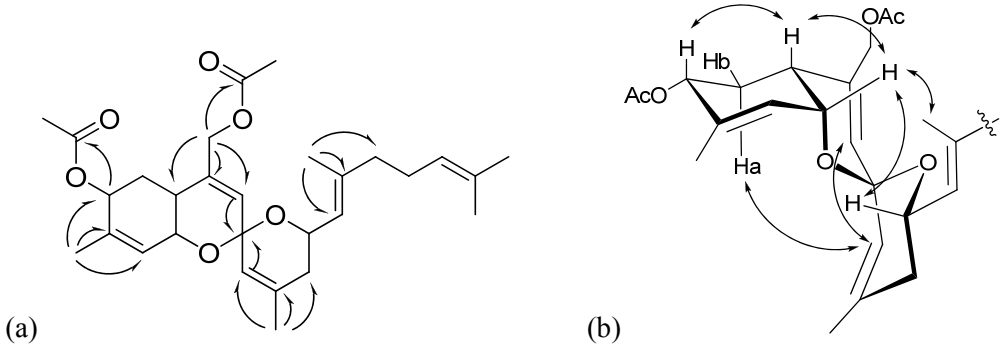
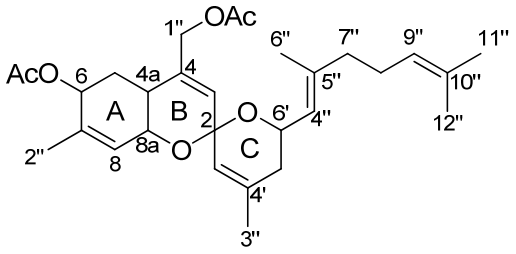


Fig 3. Key (a) HMBC and (b) NOE correlations of **1**.

The stereochemistry of **1** was established by the ROESY experiment and the coupling constants, together with comparison with the proton and carbon chemical shifts of phorbaketal B. The NOE correlations of **1** as displayed in Fig. 3 (b) were identical to those of those of phorbaketal B. However, two closely overlapped signals (2.14 ppm on H-5b and 2.13 ppm on H-4a) made it difficult to assign the NOE correlation with the proton at 5.35 ppm. Instead, the configuration of H-5 was enabled by the coupling pattern of the H-5a with three large coupling constant values. This indicates that H-5a was configured in the *anti* position with two vicinal protons; H-4a and H-6. The stereochemistry of the proton at 5.35

Table 1. NMR spectral data for compound **1** in CD_3OD at 500MHz NMR.



	δ_C	δ_H(*J* in Hz)
2	95.4, s	
3	127.8, d	5.56, br s
4	139.0, s	
4a	34.2, d	2.13, m
5	29.2, t	a 1.55, dd (13.7, 12.5, 9.8); b 2.14, m
6	73.1, d	5.35, m
7	141.5, s	
8	125.3, d	5.64, dd (5.3, 1.7)
8a	64.8, d	4.28, m
3′	123.1, d	5.22, br s
4′	138.7, s	
5′	36.4, t	a 1.83, dd (17.5, 3.3); b 2.01, dm (11.1)
6′	66.7, d	4.70, ddd (11.1, 8.2, 3.3)
1″	65.8, t	a 4.62, dd (13.5, 1.6); b 4.56, dd (13.5, 1.2)
2″	19.0, q	1.72, s
3″	22.9, q	1.75, s
4″	125.0, d	5.20, d (8.2)
5″	142.1, s	
6″	16.9, q	1.74, s
7″	40.6, t	2.04, t (7.6)
8″	27.5, t	2.12, m
9″	125.0, d	5.11, m
10″	132.5, s	
11″	25.9, q	1.67, s
12″	17.8, q	1.60, s
6-OAc		
C=O	172.3, s	
CH_3	20.9, q	2.07, s
1″-OAc		
C=O	172.4, s	
CH_3	20.7, q	2.06, s

ppm was assigned as *R* form. Therefore, the structure of **1** was determined as ((2*S*,4a*R*,6*R*,6'*S*,8a*R*)-6-acetoxy-6'-((*E*)-2,6-dimethylhepta-1,5-dienyl)-4',7-dimethyl-4a,5,5',6,6',8a-hexahydrospiro[chromene-2,2'-pyran]-4-yl)methyl acetate. This compound showed a mild cytotoxicity effect against hepatoma cancer HepG2 with an IC50 value of 10.1μg/mL.

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