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Gagunins, highly oxygenated diterpenoids from the sponge *Phorbas* sp.

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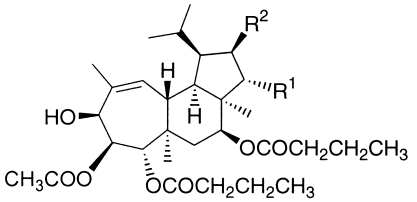
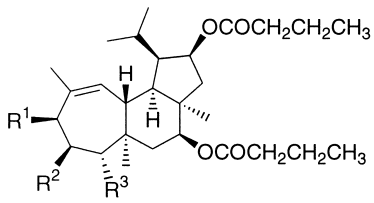
Abstract—Gagunins A–G, seven new diterpenoids were isolated from the sponge *Phorbas* sp. collected from Gagu-Do, Korea. The carbon skeleton of these highly oxygenated metabolites was determined to be an unusual 10,13-bis-*epi*-homoverrucosane on the basis of combined chemical and spectral methods. Stereochemical assignments of these diterpenoids were accomplished by extensive ROESY and 1D NOESY experiments. The novel compounds exhibited significant cytotoxicity with values ranging from 0.03 to 51 μg/mL, toward a human leukemia cell-line (K-562). © 2002 Elsevier Science Ltd. All rights reserved.

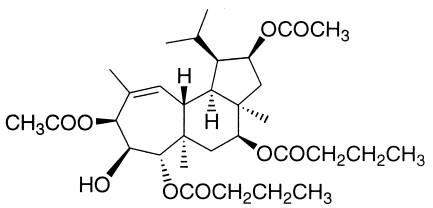
Sponges have produced a wide variety of biologically active and structurally unique secondary metabolites.[1] Of the sponge-derived natural products, terpenoids and mixed biogenetic compounds containing polyprenyl moieties are particularly abundant within the orders Dictyoceratida and Dendroceratida.[2] Several compounds from these structural classes possess diverse carbon skeletons and functionalities as well as potent bioactivities which demonstrated these sponge orders to be attractive targets for biomedical research.[1,3,4] In the course of our continuing search for bioactive natural products from marine organisms from Korea, we encountered the dark-red planar sponge *Phorbas* sp. (order Poecilosclerida, suborder Myxilina, family Anchinoidae) whose crude extract displayed significant cytotoxicity toward the human leukemia cell-line K562 (LC_{50}<1 μg/mL) and also brine-shrimp lethality (LC_{50} 36 ppm). We describe herein the structures and bioactivity of gagunins A–G (**1**–**7**), highly oxygenated diterpenoids of a new structural class. The planar carbon framework of these compounds is identical to homoverrucosane from a number of liverwort metabolites.[5–8] However, this differs from the other compounds in the stereochemistry at the juncture between 6- and 5-membered rings. In addition, a particularly unique feature of the gagunins is the presence of diverse oxygenated functionalities on the carbon framework.



	R^1	R^2
1	$OCOCH_2CH_2CH_3$	$OCOCH_2CH(CH_3)_2$
2	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$
3	$OCOCH_3$	$OCOCH_2CH_2CH_3$



	R^1	R^2	R^3
4	OH	$OCOCH_3$	$OCOCH_2CH_2CH_3$
5	$OCOCH_3$	$OCOCH_2CH_2CH_3$	OH
6	$OCOCH_3$	OH	$OCOCH_2CH_2CH_3$



7

Keywords: marine metabolites; sponge; 10,13-bis-*epi*-homoverrucosane; cytotoxicity.

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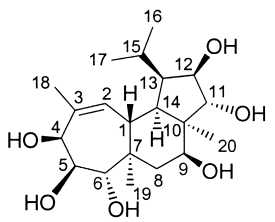
Table 1. ^{1}H and ^{13}C NMR assignments for **1** in $CDCl_3$

Position	^{1}H[a]	^{13}C	HMBC[b]	NOE[c]
1	3.46, 1H, dd (*J*=10.7, 5.9 Hz)	36.5 d	C-2, C-3, C-6, C-7, C-13, C-19	H-6, H-15
2	5.44, 1H, br d (*J*=5.9 Hz)	137.2 d	C-4, C-14	H-14, H-15, H-17, H-18
3		131.9 s		
4	4.11, 1H, br s	74.4 d	C-2, C-3, C-5, C-6, C-18	H-5, H-18
5	4.93, 1H, d (*J*=10.3 Hz)	72.6 d	C-4, C-6, OAc(C-1)	H-4, H-19
6	5.43, 1H, d (*J*=10.3 Hz)	77.9 d	C-1, C-4, C-5, C-7, C-19, 6-bu(C-1)	H-1, H-8β
7		44.7 s		
8	1.64(α), 1H, m	36.4 t	C-1, C-6, C-9, C-19	H-9, H-19
	1.50(β), 1H, dd (*J*=12.7, 12.2 Hz)			H-6, H-11
9	4.87, 1H, dd (*J*=12.2, 3.9 Hz)	74.0 d	C-10, C-11, C-20, 9-bu(C-1)	H-8α, H-19, H-20
10		47.5 s		
11	5.44, 1H, br s	78.8 d	C-9, C-10, C-12, C-13, C-20, 11-bu(C-1)	H-8β
12	5.02, 1H, br d (*J*=5.4 Hz)	80.4 d	C-10, C-11, C-14, *i*val(C-1)	H-13, H-16
13	2.16, 1H, m	52.4 d		H-12, H-14, H-17, H-20
14	1.98, 1H, dd (*J*=10.7, 4.9 Hz)	53.3 d	C-1, C-2, C-10–13, C-15	H-2, H-13, H-19, H-20
15	1.86, 1H, m	23.7 d		H-1, H-2
16	0.80, 3H, d (*J*=6.3 Hz)	21.7 q	C-13, C-15, C-17	H-12
17	0.96, 3H, d (*J*=5.9 Hz)	23.4 q	C-13, C-15, C-16	H-2, H-13, H-18
18	1.82, 3H, br s	24.0 q	C-2–4	H-2, H-4, H-17
19	1.00, 3H, s	14.1 q	C-1, C-6–8	H-5, H-8α, H-9, H-14
20	1.12, 3H, s	22.5 q	C-9–11, C-14	H-9, H-13, H-14
(5-acetoxyl)				
C-1		170.0 s		
C-2	2.03, 3H, s	21.1 q	OAc(C-1)	
(6-butyroxyl)				
C-1		172.3 s		
C-2	2.21, 2H, m	36.2 t	6-bu(C-1, C-3, C-4)	
C-3	1.60, 2H, m	18.4 t	6-bu(C-1, C-2)	
C-4	0.91, 3H, t (*J*=7.3 Hz)	13.8 q	6-bu(C-2, C-3)	
(9-butyroxyl)				
C-1		173.3 s		
C-2	2.12, 2H, m	36.3 t	9-bu(C-1, C-3, C-4)	
C-3	1.54, 2H, m	18.4 t	9-bu(C-1, C-4)	
C-4	0.88, 3H, t (*J*=7.3 Hz)	13.5 q	9-bu(C-2, C-3)	
(11-butyroxyl)				
C-1		170.7 s		
C-2	2.30, 1H, m; 2.18, 1H, m	36.3 t	9-bu(C-1)	
C-3	1.65, 2H, m	18.4 t	9-bu(C-1)	
C-4	0.96, 3H, t (*J*=7.3 Hz)	13.8 q	9-bu(C-2, C-3)	
(12-isovaleroxyl)				
C-1		172.0 s		
C-2	2.31, 1H, m; 2.18, 1H, m	43.5 t	*I*val(C-1)	
C-3	2.17, 1H, m	26.0 d		
C-4	1.04, 3H, d (*J*=5.9 Hz)	22.5 q	*I*val(C-2, C-3, C-5)	
C-5	1.02, 3H, d (*J*=5.9 Hz)	22.4 q	*i*val(C-2–4)	

Measured at 500 and 125 MHz, respectively.
[a] Multiple means that coupling patterns were not accurately measured due to the overlapping of proton signals.
[b] bu and *i*val denote butyroxyl and isovaleroxyl, respectively.
[c] Observed by ROESY and 1D NOESY experiments.



8

1. Results and discussion

The sponge *Phorbas* sp. was collected at 20–25 m depth by hand using SCUBA off the coast of Gagu-Do (Island), South-west Korea. The lyophilized specimens were repeatedly extracted with MeOH and CH_2Cl_2, respectively. The crude extracts were combined and the solvents removed under vacuum. The crude extract was then fractionated employing solvent-partitioning. Bioactivity-guided separation of the moderately polar fractions was next accomplished by C_{18} reversed-phase vacuum flash chromatography followed by reversed-phase and silica HPLC to afford gagunins A–G (**1**–**7**).

Gagunin A (**1**) was isolated as a white solid which analyzed for $C_{39}H_{62}O_{11}$ by combined HRFABMS and ^{13}C NMR spectrometry. The ^{13}C NMR spectrum of this compound displayed several signals of oxygen-bearing carbons at δ, ~170 and 85–70. Strong absorption bands at 1735 and 3450 cm^{-1} in the IR spectrum suggested that the oxygenated functionalities of **1** were indeed ester and

hydroxyl groups. The chemical shifts and splitting patterns of the proton signals in the lower region (δ, 5.5–3.3) of the ^{1}H NMR spectrum revealed that the oxygenated functionality of **1** was unrelated to a sugar moiety. Also present in the ^{13}C NMR spectrum were carbon signals for a trisubstituted double bond; δ, 137.2 (CH); 131.9 (C). Consideration of spectral data, in conjunction with the nine degree of unsaturation inherent in the molecular formula, showed that gagunin A possessed three rings.

Given this information, the gross structure of gagunin A was then determined by a combination of ^{1}H COSY, TOCSY, gradient HSQC (*g*HSQC), and gradient HMBC (*g*HMBC) NMR experiments. The *g*HSQC NMR data allowed the proton-bearing carbons and their protons to be assigned. The ^{1}H COSY and TOCSY NMR data allowed proton spin systems to be traced throughout the molecule. Beginning with the methyl signals of an isopropyl group at δ 0.96 (3H, d, J=5.9 Hz) and 0.80 (3H, d, J=6.3 Hz) in the ^{1}H NMR spectra, proton couplings were observed to several other signals including those at δ 5.44 (1H, br d, J=5.9 Hz), 5.44 (1H, br s), and 5.02 (1H, br d, J=5.4 Hz) (Table 1). Proton signals from three oxygen-bearing methines at δ 5.43 (1H, d, J=10.3 Hz), 4.93 (1H, d, J=10.3 Hz), and 4.11 (1H, br s) were coupled in a linear array. Also illustrated was an isolated spin system which consisted of the proton signals at δ 4.87 (1H, dd, J=12.2, 3.9 Hz), 1.64 (1H, m), and 1.50 (1H, dd, J=12.7, 12.2 Hz).

The partial structures was established by detailed interpretation of the *g*HMBC NMR data. Long-range correlations between the upfield methyl protons and neighboring carbons were crucial to define the ring junctures and to locate the oxygenated carbons. A methyl proton at δ 1.12 exhibited long-range correlations with the carbons at δ 78.8 (CH), 74.0 (CH), 53.3 (CH), and 47.5 (C), implying that the former three methine carbons were connected to the latter quaternary carbon. Combined with the results of proton coupling analysis, these data allowed the construction of a 5-membered ring substituted with an isopropyl group; δ 23.7 (CH), 23.4 (CH_3), 21.7 (CH_3). The same ring juncture was defined by long-range correlations of the methyl proton at δ 1.00 with carbons at δ 77.9 (CH), 44.7 (C), 36.5 (CH), and 36.4 (CH_2). In addition, long-range correlations were observed between the vinyl methyl proton at δ 1.82 and the carbons at δ 137.2 (CH), 131.9 (C), and 74.4 (CH). Since the proton at δ 3.46, attached to the methine carbon at δ 36.5, showed direct proton–proton coupling (J=5.9 Hz) with the olefinic proton at δ 5.44 (δ_C 137.2), these two-dimensional (2D) NMR data were consistent with a 7-membered ring at the terminus of the molecule. Finally the presence of a 6-membered ring in the central core of the molecule was also secured by long-range correlations of the protons at δ 4.87, 1.64, and 1.50 with neighboring carbons. Thus, the planar carbon framework of **1** was determined to be identical to that defined for the homoverrucosane skeleton.

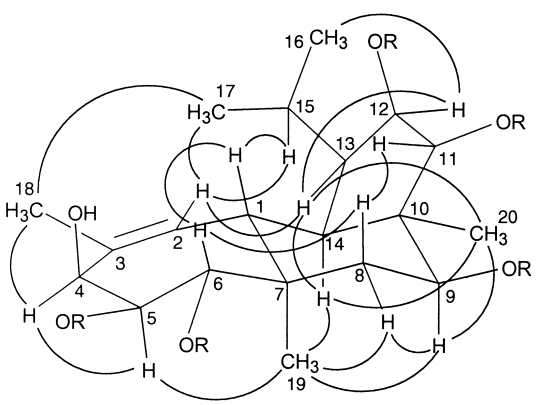


Figure 1. Selected NOE correlations of compound **1**.

Gagunin A (**1**) possessed oxygenated functionalities at C-4–6, C-9, C-11, and C-12. The identification and the assignment of these functionalities was accomplished by combined 2D NMR analyses. A series of proton couplings, including signals of the methyl protons at δ 1.04 (3H, d, J=5.9 Hz) and 1.02 (3H, d, J=5.9 Hz), revealed the presence of an isovaleroxyl group. Tracing of the proton couplings beginning with the upfield methyl protons at δ 0.96 (3H, t, J=7.3 Hz), 0.91 (3H, t, J=7.3 Hz), and 0.88 (3H, t, J=7.3 Hz) showed the presence of three butyroxyl groups. An isolated methyl signal at δ 2.03 (3H, s) was assigned to an acetoxyl group. Gradient HMBC NMR correlations of the five ester carbons (δ_C ~170) with oxymethine protons allowed the acetoxyl group to be positioned at C-5, the isovaleroxyl group at C-12, and three butyroxyl groups at C-6, C-9, and C-11. The remaining hydroxyl group was assigned at C-4. Thus, the planar structure of **1** was determined to be 5-acetoxy-6,9,11-tributyroxy-4-hydroxy-12-isovaleroxyhomoverrucosa-2-ene.

Gagunin A (**1**) possessed 11 asymmetric carbon centers at C-1, C-4–7 and C-9–14. The stereochemistries at these centers were assigned on the basis of combined ROESY and 1D NOESY experiments (Fig. 1). The H-1 proton at the A/B ring junction displayed ROESY cross-peak correlations with H-6, which in turn correlated with H-8β (δ, 1.50). Conversely, the H-19 methyl protons at the same ring junction showed cross-peak correlations with H-5, H-8β (δ 1.64), H-9, and H-14. Accordingly the A/B ring junction was assigned a *trans* orientation and the former and latter protons were assigned to β- and α-orientations to the AB ring plane, respectively. The α-orientation of H-4 was secured by its NOE correlation with H-5 and a lack of similar correlation with H-6, as well as its small vicinal proton coupling constant ($J_{4,5}$<1 Hz). The H-20 methyl protons at the B/C ring junction displayed NOE correlations with H-9, H-13 and H-14. The H-13 methine proton showed additional correlations with H-12 and H-14. Coupled with the NOE correlations between H-9 and H-20 and also between H-14 and H-19, these data suggested a *cis* B/C ring junction and β-orientations for H-12, H-13, H-14, and H-20. The H-11 methine proton, which did not show NOE correlations with any proton with the C ring, was assigned a β-orientation on the basis of its strong NOE correlation with H-8β. The H-16 isopropyl methyl protons exhibited strong NOE correlation with H-12 while the H-17 methyl protons displayed correlations with H-2 and H-18. A three-dimensional molecular model for **1** revealed that steric overcrowding with the bulky isovaleroxyl group at the adjacent C-12 prevented free rotation of the isopropyl group. Restricted rotation would result in the significant differentiation between the H-16 (δ 0.80, J=6.3 Hz) and

H-17 (δ 0.96, J=5.9 Hz) methyl groups. Thus, the relative configurations of the asymmetric carbon centers of **1** were assigned as 1R^*, 4S^*, 5S^*, 6S^*, 7R^*, 9S^*, 10R^*, 11R^*, 12R^*, 13R^*, 14S^*.

The structure of gagunin A was confirmed by chemical modification. Treatment with KOH converted **1** to the perhydroxyl derivative **8**. In **8**, all of the NMR signals of the ester chains which complicated the upfield regions in the ^{1}H and ^{13}C NMR data of **1** were removed. Accordingly, the NMR spectra of **8** were very concise and 2D NMR correlations were clarified. In addition to the NOE correlations obtained for **1**, new key correlations were also observed; H-1 and H-11, H-12 and H-14, H-12 and H-20. These data were supportive for the β- and α-orientations of H-11 and H-12, respectively. Thus, the structure of gagunin A (**1**) was determined as a diterpenoid of the homoverrucosane class. A literature survey revealed that diterpenoids possessing verrucosane and related carbon skeletons have been found mainly in liverworts.[5–8] Among marine organisms, metabolites possessing the verrucosane and neoverrucosane carbon skeletons have been isolated from the tropical sponge *Axynissa aplysinoides*.[9] However, the carbon skeleton of gagunin A, 10,13-bis-*epi*-homoverrucosane, differs from the other metabolites in its stereochemistry of asymmetric carbon centers. An additional distinctive feature of this compound is the presence of diverse oxygenated functionalities.

The molecular formula of gagunin B (**2**) was deduced as $C_{38}H_{60}O_{11}$ by HRFABMS and ^{13}C NMR spectrometry. The NMR spectral data of this compound were very similar to those obtained for **1**. The only noticeable difference in the ^{13}C NMR spectrum was the disappearance of signals for a methyl and a methine carbon in the upfield region. Instead, signal of a new methylene carbon appeared at δ 36.3 in **2**. Corresponding changes were also observed in the ^{1}H NMR spectra in which signals at δ 1.04 (3H, d, J=5.9 Hz) and 1.02 (3H, d, J=5.9 Hz) were replaced by a new signal at δ 1.01 (3H, t, J=7.3 Hz). These spectral differences were readily accommodated by the replacement of the isovaleroxyl group, substituted at C-12 of **1**, with a butyroxyl group in **2**. This proposal was confirmed by subsequent interpretation of combined 2D NMR data.

The related metabolite, gagunin C (**3**), was isolated as a white solid that analyzed for $C_{36}H_{56}O_{11}$ by HRFABMS and ^{13}C NMR spectral methods. The NMR spectra of this compound were highly compatible with those of **1** and **2**. Examination of the NMR spectral data revealed that one of the butyroxyl groups of **2** was replaced by an acetoxyl group in **3**; δ_C 21.2/20.8 (CH_3), δ_H 2.03 (3H, s). The *g*HMBC NMR spectrum displayed three bond correlations of the ester carbon at δ 168.2 (C) with the protons at δ 5.41 (1H, br s, H-11) and 2.03 (3H, s). Thus, the structure of gagunin C (**3**) was defined as a derivative of gagunin B (**2**) containing an acetoxyl group at C-11.

The molecular formula of gagunin D (**4**) was assigned as $C_{34}H_{54}O_9$ on the basis of the results of HRFABMS and interpretation of ^{13}C NMR data. Preliminary examination of the ^{1}H and ^{13}C NMR spectra of this compound revealed that one of the butyroxyl groups found in **2** had disappeared. A combination of ^{1}H COSY and TOCSY NMR experiments showed that H-11 at δ 5.42 (1H, s) was replaced by a new proton at δ 1.88 (2H, br d, J=4.9 Hz). Supporting evidence was provided by *g*HMBC data which showed long-range correlations between the newly added methylene protons and C-9, C-10, C-12–14, and C-20. The corresponding carbon at δ 40.5 (CH_2) also displayed long-range correlations with H-9, H-14, and H-20. Thus, the structure of **4** was determined as the 11-desbutyroxyl derivative of **2**.

The molecular formula of gagunin E (**5**) was established as $C_{34}H_{54}O_9$, identical to that of **4**, on the basis of combined HRFABMS and interpretation of ^{13}C NMR data. The ^{1}H and ^{13}C NMR data of this compound were also very similar to those of **4**. However, detailed examination of the NMR data revealed that signals of several protons and carbons of A ring were considerably shifted between **4** and **5**. On the other hand, a combination of the ^{1}H COSY, TOCSY, and *g*HSQC experiments showed the same homo- and hetero-NMR correlations between these compounds, implying that the structural difference occurred by a shift of substituents within A ring. The downfield shift of H-4 at δ 5.10 (1H, d, J=2.4 Hz), as well as its *g*HMBC correlations with C-2, C-3, C-5, and C-18 revealed the attachment of a carboxylic group at this location. The substituent was determined to be an acetoxyl group on the basis of long-range correlations of the carboxylic carbon at δ 170.0 (C) with H-4 and the methyl proton at δ 2.10 (3H, s). Similarly the attachment of a butyroxyl group at C-5 was secured by long-range correlations of a carboxylic carbon at δ 173.6 (C) with H-5 at δ 4.94 (1H, dd, J=9.8, 2.4 Hz) and the butyroxyl protons at δ 2.33 (2H, t, J=7.3 Hz) and 1.66 (2H, hex, J=7.3 Hz). In addition, the upfield shift of H-6 at δ 3.74 (1H, d, J=9.8 Hz), coupled with *g*HMBC correlations of the corresponding carbon at δ 79.5 (CH) with H-1, H-4, H-5, H-8, and H-19, suggested placement of a hydroxyl group at C-6.

The molecular formula of gagunin F (**6**) was established as $C_{34}H_{54}O_9$, also identical to those of **4** and **5**, by HRFABMS and ^{13}C NMR spectrometry. The ^{1}H and ^{13}C NMR spectral data of this compound were also very similar to those of **4** and **5**. An observation of the same proton–proton coupling patterns throughout the entire molecule revealed that the structural variation occurred only on the location of the oxygenated functionalities. A combination of the chemical shifts analysis of key protons and 2D NMR data, as described for the structure determination of **5**, positioned butyroxyl and hydroxyl groups at C-6 and C-5, respectively, for **6** while those at the remaining locations were identical to **5**. ROESY and 1D NOESY NMR experiments assigned the stereochemistry of gagunin F was identical to those of the other metabolites.

Lastly, gagunin G (**7**) was isolated as a white solid that analyzed for $C_{32}H_{50}O_9$ by combined HRFABMS and ^{13}C NMR spectral methods. Based upon the results of 1D and 2D NMR experiments, it was apparent that this compound was also an analog of other gagunins with the structural variation derived from the locations of the oxygenated substituents. Gradient HMBC correlations of the carbon at δ 170.5 (C) with H-12 at δ 5.15 (1H, br d, J=6.3 Hz) and a methyl proton at δ 2.25 (3H, s) positioned an acetoxyl group

at C-12. The nature of substituents at other locations, as well as the stereochemistry at asymmetric centers, was identical to those of **6**.

The crude extract of *Phorbas* sp. exhibited significant cytotoxicity toward the human leukemia cell-line K562. The same measurement using pure compounds revealed that gagunins were the causative metabolites of bioactivity with LC_{50} values of 50.1, 10.4, 0.71, 0.13, 0.03, 0.11, and 2.0 μg/mL for **1**–**7**, respectively. The significant differences in cytotoxicity, up to three orders of magnitude, among these metabolites is quite remarkable. It is also noteworthy that compounds **1** and **2**, containing a bulky substituent at C-11, are far less active than others possessing either an acetoxyl group or hydrogen at the same position. Conversely, compound **8**, the synthetic perhydroxyl derivative, was inactive with LC_{50} value of >100 μg/mL toward K562.

2. Experimental

2.1. General experimental procedures

Melting points were measured on a Fisher–Jones Apparatus and are uncorrected. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. IR spectra were recorded on a Mattson GALAXY spectrophotometer. NMR spectra were recorded in $CDCl_3$ and CD_3OD solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectral data were provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

2.2. Animal material

Specimens of *Phorbas* sp. (sample number 00SH-2) were collected by hand using SCUBA at 20–25 m depth offshore of Gagu-do (Island), Korea in July, 2000. The sponge had irregular surfaces composed of mountain-like peaks. Oscules were rare and the texture was very soft. The color in life was dark red. In the skeleton, megascleres were tornotes (295–410×7–10 μm), small acanthostyles (145–185×8–10 μm), and large acanthostyles (300–420×9–11 μm), and microscleres were isochelas (25–30 μm). This sponge had thicker megascleres than Pulitzer-Finali's specimen from Bay of Naples.[10] The growth form of this specimen was a thick mass while that from Naples was incrusting. The voucher specimen (registry no. Spo. 37) is on deposit at the Natural History Museum, Hannam University, Korea under the curatorship of C. J. S.

2.3. Extraction and isolation

The fresh collection was immediately frozen and kept at −25°C until chemically investigated. The specimens were lyophilized (dry wt 1.8 kg), macerated, and repeatedly extracted with MeOH (1 L×3) and CH_2Cl_2 (1 L×2). The combined crude extract (18.9 g) was partitioned between *n*-BuOH and H_2O, then the former layer re-partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was evaporated to dryness in vacuo and the residue (8.81 g) was partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (3.93 g) was subjected to C_{18} reversed-phase vacuum flash chromatography using gradient mixtures of MeOH and H_2O as eluents (elution order 50, 40, 30, 20, 10% aqueous MeOH, 100% MeOH). The fraction (490 mg) eluted with 10% aqueous MeOH was dried and separated by reversed-phase HPLC (YMC-ODS-A column, 20% aqueous MeOH) to afford in the order of elution, **7**, **3**, **5**, **6**, **4**, **2**, and **1** as white solids. Proton NMR analysis revealed that compounds **1** and **2** were pure while others contained impurities. Purifications of the remaining metabolites were then accomplished by silica HPLC (YMC-silica column, 25% EtOAc in *n*-hexane for **3**–**6**, 30% EtOAc in *n*-hexane for **7**). The purified metabolites were isolated in the following amounts: 20.4, 59.0, 1.5, 5.9, 3.1, 5.8, and 2.9 mg for **1**–**7**, respectively.

2.3.1. Gagunin A (1). Amorphous solid; mp 88–90°C; $[\alpha]_D^{25}$=48.6° (*c* 0.71, MeOH); IR (KBr) ν_{max} 3450 (br), 2965, 1735, 1460, 1370, 1245 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 729.4189 $[M+Na]^+$ (calcd for $C_{39}H_{62}O_{11}Na$, 729.4190, Δ −0.1 mmu).

2.3.2. Gagunin B (2). Amorphous solid; mp 76–78°C; $[\alpha]_D^{25}$=55.3° (*c* 0.65, MeOH); IR (KBr) ν_{max} 3450 (br), 2965, 1735, 1460, 1375, 1245 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.43 (1H, d, *J*=10.3 Hz, H-6), 5.42 (1H, br d, *J*=5.9 Hz, H-2), 5.42 (1H, s, H-11), 5.03 (1H, d, *J*=5.9 Hz, H-12), 4.94 (1H, dd, *J*=10.3, 1.0 Hz, H-5), 4.87 (1H, dd, *J*=12.7, 3.9 Hz, H-9), 4.11 (1H, br s, H-4), 3.48 (1H, dd, *J*=10.3, 5.9 Hz, H-1), 2.42 (1H, dt, *J*=15.4, 7.3 Hz, H-2 (12-butyroxyl)), 2.33 (1H, dt, *J*=15.4, 7.3 Hz, H-2 (12-butyroxyl)), 2.30 (1H, m, H-2 (11-butyroxyl)), 2.22 (3H, m, H-2 (6-butyroxyl, 11-butyroxyl)), 2.17 (1H, m, H-13), 2.14 (2H, m, H-2 (9-butyroxyl)), 2.03 (3H, s, OAc), 1.98 (1H, dd, *J*=10.3, 5.4 Hz, H-14), 1.86 (1H, m, H-15), 1.83 (3H, br s, H-18), 1.74 (2H, m, H-3 (12-butyroxyl)), 1.66 (2H, m, H-3 (11-butyroxyl)), 1.64 (1H, m, H-8α), 1.61 (2H, m, H-3 (6-butyroxyl)), 1.55 (2H, hex, *J*=7.3 Hz, H-3 (9-butyroxyl)), 1.50 (1H, dd, *J*=12.7, 12.7 Hz, H-8β), 1.12 (3H, s, H-20), 1.01 (3H, t, *J*=7.3 Hz, H-4 (12-butyroxyl)), 1.01 (3H, s, H-19), 0.97 (3H, t, *J*=7.3 Hz, H-4 (11-butyroxyl)), 0.96 (3H, d, *J*=5.9 Hz, H-17), 0.92 (3H, t, *J*=7.3 Hz, H-4 (6-butyroxyl)), 0.88 (3H, t, *J*=7.3 Hz, H-4 (9-butyroxyl)), 0.81 (3H, d, *J*=6.4 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 173.3 (C, C-1 (9-butyroxyl)), 172.5 (C, C-1 (12-butyroxyl)), 172.4 (C, C-1 (6-butyroxyl)), 170.7 (C, C-1 (11-butyroxyl)), 170.0 (C, OAc), 137.3 (CH, C-2), 131.9 (C, C-3), 80.4 (CH, C-12), 78.8 (CH, C-11), 77.9 (CH, C-6), 74.4 (CH, C-4), 74.0 (CH, C-9), 72.6 (CH, C-5), 53.3 (CH, C-14), 52.5 (CH, C-13), 47.5 (C, C-10), 44.7 (C, C-7), 36.5 (CH, C-1), 36.4 (CH_2, C-8), 36.3 (CH_2×2, C-2 (6-butyroxyl, 11-butyroxyl)), 36.2 (CH_2×2, C-2 (9-butyroxyl, 12-butyroxyl)), 24.0 (CH_3, C-18), 23.7 (CH, C-15), 23.4 (CH_3, C-17), 22.6 (CH_3, C-20), 21.7 (CH_3, C-16), 21.2 (CH_3, OAc), 18.6 (CH_2, C-3 (12-butyroxyl)), 18.4 (CH_2×3, C-3 (6-butyroxyl, 9-butyroxyl, 11-butyroxyl)), 14.1 (CH_3, C-19), 13.8 (CH_3×2, C-4 (6-butyroxyl, 12-butyroxyl)), 13.7 (CH_3, C-4 (11-butyroxyl)), 13.5 (CH_3, C-4 (9-butyroxyl)); HRFABMS *m/z* 715.4036 $[M+Na]^+$ (calcd for $C_{38}H_{60}O_{11}Na$, 715.4033, Δ +0.3 mmu).

2.3.3. Gagunin C (3). Amorphous solid; mp 63–66°C; $[\alpha]_D^{25}$=54.1° (*c* 0.04, MeOH); IR (KBr) ν_{max} 3450 (br),

2960, 1735, 1460, 1370, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.44 (1H, d, J=10.3 Hz, H-6), 5.43 (1H, br d, J=5.9 Hz, H-2), 5.41 (1H, br s, H-11), 5.05 (1H, d, J=6.4 Hz, H-12), 4.94 (1H, dd, J=10.3, 1.5 Hz, H-5), 4.88 (1H, dd, J=12.7, 4.4 Hz, H-9), 4.11 (1H, br s, H-4), 3.47 (1H, dd, J=10.3, 5.9 Hz, H-1), 2.42 (1H, dt, J=15.1, 7.3 Hz, H-2 (12-butyroxyl)), 2.33 (1H, dt, J=15.1, 7.3 Hz, H-2 (12-butyroxyl)), 2.23 (2H, m, H-2 (6-butyroxyl)), 2.17 (1H, m, H-13), 2.15 (2H, m, H-2 (9-butyroxyl)), 2.03 (6H, s, 5-OAc, 11-OAc), 1.98 (1H, dd, J=10.3, 5.4 Hz, H-14), 1.87 (1H, m, H-15), 1.83 (3H, br s, H-18), 1.74 (2H, hex, J=7.3 Hz, H-3 (12-butyroxyl)), 1.64 (1H, dd, J=12.7, 4.4 Hz, H-8α), 1.61 (2H, m, H-3 (6-butyroxyl)), 1.57 (2H, hex, J=7.3 Hz, H-3 (9-butyroxyl)), 1.51 (1H, dd, J=12.7, 12.7 Hz, H-8β), 1.12 (3H, s, H-20), 1.02 (3H, t, J=7.3 Hz, H-4 (12-butyroxyl)), 1.01 (3H, s, H-19), 0.96 (3H, d, J=5.9 Hz, H-17), 0.92 (3H, t, J=7.3 Hz, H-4 (6-butyroxyl)), 0.89 (3H, t, J=7.3 Hz, H-4 (9-butyroxyl)), 0.81 (3H, d, J=6.4 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 173.2 (C, C-1 (9-butyroxyl)), 172.6 (C, C-1 (12-butyroxyl)), 172.4 (C, C-1 (6-butyroxyl)), 170.0 (C, 5-OAc), 168.2 (C, 11-OAc), 137.2 (CH, C-2), 131.9 (C, C-3), 80.3 (CH, C-12), 79.2 (CH, C-11), 77.9 (CH, C-6), 74.4 (CH, C-4), 73.9 (CH, C-9), 72.6 (CH, C-5), 53.2 (CH, C-14), 52.4 (CH, C-13), 47.4 (C, C-10), 44.7 (C, C-7), 36.49 (CH, C-1), 36.46 (CH_2, C-8), 36.3 (CH_2×2, C-2 (9-butyroxyl, 12-butyroxyl)), 36.2 (CH_2, C-2 (6-butyroxyl)), 24.0 (CH_3, C-18), 23.7 (CH, C-15), 23.4 (CH_3, C-17), 22.5 (CH_3, C-20), 21.7 (CH_3, C-16), 21.2 (CH_3, OAc), 20.8 (CH_3, OAc), 18.6 (CH_2, C-3 (12-butyroxyl)), 18.4 (CH_2×2, C-3 (6-butyroxyl, 9-butyroxyl)), 14.1 (CH_3, C-19), 13.8 (CH_3, C-4 (6-butyroxyl)), 13.7 (CH_3, C-4 (12-butyroxyl)), 13.6 (CH_3, C-4 (9-butyroxyl)); HRFABMS m/z 687.3718 $[M+Na]^+$ (calcd for $C_{36}H_{56}O_{11}Na$, 687.3720, Δ −0.2 mmu).

2.3.4. Gagunin D (4). Amorphous solid; mp 73–75°C; $[\alpha]_D^{25}$=51.3° (c 0.12, MeOH); IR (KBr) ν_{max} 3450 (br), 2965, 1730, 1460, 1240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.47 (1H, br d, J=5.9 Hz, H-2), 5.42 (1H, d, J=10.3 Hz, H-6), 5.14 (1H, dt, J=4.4, 4.9 Hz, H-12), 4.96 (1H, dd, J=10.3, 1.5 Hz, H-5), 4.92 (1H, dd, J=12.2, 3.9 Hz, H-9), 4.12 (1H, br s, H-4), 3.50 (1H, dd, J=9.8, 5.9 Hz, H-1), 2.37 (1H, dt, J=12.8, 7.3 Hz, H-2 (12-butyroxyl)), 2.34 (1H, dt, J=12.8, 7.3 Hz, H-2 (12-butyroxyl)), 2.26 (1H, m, H-2 (6-butyroxyl)), 2.22 (2H, t, J=7.3 Hz, H-2 (9-butyroxyl)), 2.20 (1H, m, H-2 (6-butyroxyl)), 2.04 (3H, s, OAc), 1.96 (1H, m, H-13), 1.94 (1H, m, H-15), 1.88 (2H, br d, J=4.9 Hz, H-11), 1.84 (3H, br s, H-18), 1.82 (1H, dd, J=9.8, 4.4 Hz, H-14), 1.73 (2H, hex, J=7.3 Hz, H-3 (12-butyroxyl)), 1.60 (4H, m, H-3 (6-butyroxyl, 9-butyroxyl)), 1.56 (1H, dd, J=12.7, 3.9 Hz, H-8α), 1.36 (1H, dd, J=12.7, 12.2 Hz, H-8β), 1.06 (3H, s, H-20), 1.01 (3H, s, H-19), 1.00 (3H, t, J=7.3 Hz, H-4 (12-butyroxyl)), 0.96 (3H, d, J=5.4 Hz, H-17), 0.93 (3H, t, J=7.3 Hz, H-4 (6-butyroxyl)), 0.92 (3H, t, J=7.3 Hz, H-4 (9-butyroxyl)), 0.86 (3H, d, J=5.9 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 173.5 (C×2, C-1 (9-butyroxyl, 12-butyroxyl)), 172.7 (C, C-1 (6-butyroxyl)), 170.0 (C, OAc), 138.1 (CH, C-2), 131.6 (C, C-3), 78.1 (CH, C-6), 76.2 (CH, C-12), 74.7 (CH, C-9), 74.5 (CH, C-4), 72.6 (CH, C-5), 55.0 (CH, C-13), 52.7 (CH, C-14), 44.8 (C, C-7), 44.7 (C, C-10), 40.5 (CH_2, C-11), 36.6 (CH_2, C-8), 36.5 (CH_2×3, C-2 (6-butyroxyl, 9-butyroxyl, 12-butyroxyl)), 36.2 (CH, C-1), 29.3 (CH_3, C-20), 23.99 (CH_3, C-18), 23.97 (CH, C-15), 23.6 (CH_3, C-17), 21.9 (CH_3, C-16), 21.2 (CH_3, OAc), 18.6 (CH_2, C-3 (12-butyroxyl)), 18.5 (CH_2, C-3 (9-butyroxyl)), 18.4 (CH_2, C-3 (6-butyroxyl)), 14.1 (CH_3, C-19), 13.8 (CH_3×2, C-4 (9-butyroxyl, 12-butyroxyl)), 13.6 (CH_3, C-4 (6-butyroxyl)); HRFABMS m/z 629.3664 $[M+Na]^+$ (calcd for $C_{34}H_{54}O_9Na$, 629.3666, Δ −0.2 mmu).

2.3.5. Gagunin E (5). Amorphous solid; mp 57–59°C; $[\alpha]_D^{25}$=161.6° (c 0.21, MeOH); IR (KBr) ν_{max} 3500 (br), 2965, 1730, 1460, 1370, 1235 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.38 (1H, br d, J=7.3 Hz, H-2), 5.16 (1H, dd, J=5.9, 5.4 Hz, H-12), 5.10 (1H, d, J=2.4 Hz, H-4), 5.01 (1H, dd, J=12.7, 3.4 Hz, H-9), 4.94 (1H, dd, J=9.8, 2.4 Hz, H-5), 3.74 (1H, d, J=9.8 Hz, H-6), 3.04 (1H, dd, J=7.8, 7.3 Hz, H-1), 2.35 (2H, m, H-2 (12-butyroxyl)), 2.33 (2H, t, J=7.3 Hz, H-2 (5-butyroxyl)), 2.23 (2H, t, J=7.3 Hz, H-2 (9-butyroxyl)), 2.15 (1H, dd, J=12.7, 3.4 Hz, H-8α), 2.10 (3H, s, OAc), 1.94 (1H, m, H-13), 1.92 (1H, d, J=16.5 Hz, H-11), 1.89 (3H, br s, H-18), 1.84 (1H, dd, J=16.5, 5.9 Hz, H-11), 1.82 (1H, dd, J=7.8, 5.9 Hz, H-14), 1.74 (2H, hex, J=7.3 Hz, H-3 (12-butyroxyl)), 1.66 (2H, hex, J=7.3 Hz, H-3 (5-butyroxyl)), 1.64 (1H, m, H-15), 1.62 (2H, hex, J=7.3 Hz, H-3 (9-butyroxyl)), 1.28 (1H, dd, J=12.7, 12.7 Hz, H-8β), 1.09 (3H, s, H-20), 1.00 (3H, t, J=7.3 Hz, H-4 (12-butyroxyl)), 0.99 (3H, s, H-19), 0.95 (3H, t, J=7.3 Hz, H-4 (5-butyroxyl)), 0.93 (3H, t, J=7.3 Hz, H-4 (9-butyroxyl)), 0.89 (3H, d, J=6.3 Hz, H-17), 0.86 (3H, d, J=6.8 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 173.6 (C, C-1 (5-butyroxyl)), 173.4 (C, C-1 (9-butyroxyl)), 173.2 (C, C-1 (12-butyroxyl)), 170.0 (C, OAc), 140.2 (CH, C-2), 130.4 (C, C-3), 79.5 (CH, C-6), 78.0 (CH, C-12), 75.1 (CH, C-4), 74.8 (CH, C-9), 72.3 (CH, C-5), 55.0 (CH, C-13), 53.8 (CH, C-14), 44.8 (C, C-7), 44.7 (C, C-10), 41.6 (CH_2, C-11), 37.5 (CH, C-1), 37.0 (CH_2, C-8), 36.8 (CH_2, C-2 (12-butyroxyl)), 36.6 (CH_2, C-2 (9-butyroxyl)), 36.3 (CH_2, C-2 (5-butyroxyl)), 30.4 (CH_3, C-20), 23.9 (CH, C-15), 23.7 (CH_3, C-18), 23.5 (CH_3, C-17), 21.6 (CH_3, C-16), 20.9 (CH_3, OAc), 18.6 (CH_2×2, C-3 (9-butyroxyl, 12-butyroxyl)), 18.4 (CH_2, C-3 (5-butyroxyl)), 13.8 (CH_3, C-19), 13.7 (CH_3×2, C-4 (9-butyroxyl, 12-butyroxyl)), 13.6 (CH_3, C-4 (5-butyroxyl)); HRFABMS m/z 629.3663 $[M+Na]^+$ (calcd for $C_{34}H_{54}O_9Na$, 629.3666, Δ −0.3 mmu).

2.3.6. Gagunin F (6). Amorphous solid; mp 59–61°C; $[\alpha]_D^{25}$=157.3° (c 0.14, MeOH); IR (KBr) ν_{max} 3500 (br), 2965, 1730, 1460, 1380, 1235 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.32 (1H, br d, J=7.8 Hz, H-2), 5.16 (1H, dd, J=5.9, 5.4 Hz, H-12), 5.11 (1H, d, J=9.8 Hz, H-6), 5.04 (1H, d, J=1.0 Hz, H-4), 4.94 (1H, dd, J=12.2, 3.4 Hz, H-9), 3.73 (1H, br d, J=9.8 Hz, H-5), 3.15 (1H, dd, J=7.8, 7.8 Hz, H-1), 2.52 (1H, dt, J=16.1, 7.3 Hz, H-2 (12-butyroxyl)), 2.38 (1H, dt, J=16.1, 7.3 Hz, H-2 (12-butyroxyl)), 2.33 (2H, t, J=7.3 Hz, H-2 (6-butyroxyl)), 2.23 (2H, t, J=7.3 Hz, H-2 (9-butyroxyl)), 2.13 (3H, s, OAc), 1.92 (1H, br d, J=16.2 Hz, H-11), 1.90 (3H, s, H-18), 1.89 (1H, m, H-13), 1.81 (2H, m, H-11, H-14), 1.74 (2H, hex, J=7.3 Hz, H-3 (12-butyroxyl)), 1.65 (1H, m, H-15), 1.63 (2H, m, H-3 (6-butyroxyl)), 1.61 (2H, m, H-3 (9-butyroxyl)), 1.59 (1H, dd, J=12.7, 3.4 Hz, H-8α), 1.24 (1H, dd, J=12.7, 12.2 Hz, H-8β), 1.08 (3H, s, H-20), 1.01 (3H, s, H-19), 1.00 (3H, t, J=7.3 Hz, H-4 (12-butyroxyl)), 0.93 (3H, t, J=7.3 Hz, H-4 (6-butyroxyl)), 0.92 (3H, t, J=7.3 Hz, H-4 (9-butyroxyl)), 0.872 (3H, d,

J=5.9 Hz, H-17), 0.867 (3H, d, J=6.8 Hz, H-16); ^{13}C NMR ($CDCl_3$) δ 174.4 (C, C-1 (6-butyroxyl)), 173.5 (C, C-1 (9-butyroxyl)), 173.4 (C, C-1 (12-butyroxyl)), 170.2 (C, OAc), 139.5 (CH, C-2), 131.1 (C, C-3), 82.3 (CH, C-6), 78.6 (CH, C-4), 77.9 (CH, C-12), 74.6 (CH, C-9), 69.1 (CH, C-5), 55.1 (CH, C-13), 53.7 (CH, C-14), 44.7 (C, C-10), 44.1 (C, C-7), 41.6 (CH_2, C-11), 37.2 (CH, C-1), 36.6 (CH_2, C-8), 36.5 (CH_2×2, C-2 (6-butyroxyl, 12-butyroxyl)), 36.3 (CH_2, C-2 (9-butyroxyl)), 30.5 (CH_3, C-20), 23.9 (CH, C-15), 23.8 (CH_3, C-18), 23.3 (CH_3, C-17), 21.5 (CH_3, C-16), 20.8 (CH_3, OAc), 18.5 (CH_2×3, C-3 (6-butyroxyl, 9-butyroxyl, 12-butyroxyl)), 15.0 (CH_3, C-19), 13.6 (CH_3×3, C-4 (6-butyroxyl, 9-butyroxyl, 12-butyroxyl)); HRFABMS m/z 629.3664 $[M]^+$ (calcd for $C_{34}H_{54}O_9Na$, 629.3666, Δ −0.2 mmu).

2.3.7. Gagunin G (7). Amorphous solid; mp 77–78°C; $[\alpha]_D^{25}$=74.9° (c 0.18, MeOH); IR (KBr) ν_{max} 3500 (br), 2960, 1735, 1455, 1380, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.32 (1H, br d, J=7.3 Hz, H-2), 5.15 (1H, br d, J=6.3 Hz, H-12), 5.13 (1H, d, J=9.3 Hz, H-6), 5.04 (1H, d, J=1.0 Hz, H-4), 4.94 (1H, dd, J=12.7, 2.9 Hz, H-9), 3.74 (1H, br d, J=9.3 Hz, H-5), 3.24 (1H, dd, J=7.3, 7.3 Hz, H-1), 2.34 (2H, t, J=7.3 Hz, H-2 (6-butyroxyl)), 2.25 (3H, s, 12-OAc), 2.23 (2H, t, J=7.3 Hz, H-2 (9-butyroxyl)), 2.13 (3H, s, 4-OAc), 1.97 (1H, d, J=16.6 Hz, H-11), 1.90 (3H, br s, H-18), 1.87 (1H, br dd, J=10.8, 5.9 Hz, H-13), 1.81 (1H, dd, J=7.3, 5.9 Hz, H-14), 1.75 (1H, dd, J=16.6, 6.3 Hz, H-11), 1.66 (2H, hex, J=7.3 Hz, H-3 (6-butyroxyl)), 1.64 (1H, m, H-15), 1.62 (2H, hex, J=7.3 Hz, H-3 (9-butyroxyl)), 1.59 (1H, dd, J=12.7, 2.9 Hz, H-8α), 1.26 (1H, dd, J=12.7, 12.7 Hz, H-8β), 1.08 (3H, s, H-20), 1.02 (3H, s, H-19), 0.94 (3H, t, J=7.3 Hz, H-4 (6-butyroxyl)), 0.92 (3H, t, J=7.3 Hz, H-4 (9-butyroxyl)), 0.88 (3H, d, J=7.3 Hz, H-16), 0.87 (3H, d, J=6.4 Hz, H-17); ^{13}C NMR ($CDCl_3$) δ 174.5 (C, C-1 (6-butyroxyl)), 173.5 (C, C-1 (9-butyroxyl)), 170.5 (C, 12-OAc), 170.1 (C, 4-OAc), 139.6 (CH, C-2), 131.1 (C, C-3), 82.4 (CH, C-6), 78.7 (CH, C-4), 78.4 (CH, C-12), 74.6 (CH, C-9), 69.0 (CH, C-5), 55.2 (CH, C-13), 53.6 (CH, C-14), 44.7 (C, C-10), 44.0 (C, C-7), 41.5 (CH_2, C-11), 37.1 (CH, C-1), 36.6 (CH_2, C-2 (9-butyroxyl)), 36.5 (CH_2, C-8), 36.3 (CH_2, C-2 (6-butyroxyl)), 30.6 (CH_3, C-20), 23.8 (CH, C-15), 23.7 (CH_3, C-18), 23.1 (CH_3, C-17), 22.0 (CH_3, 12-OAc), 21.5 (CH_3, C-16), 20.8 (CH_3, 4-OAc), 18.5 (CH_2×2, C-3 (6-butyroxyl, 9-butyroxyl)), 15.1 (CH_3, C-19), 13.7 (CH_3, C-4 (6-butyroxyl)), 13.6 (CH_3, C-4 (9-butyroxyl)); HRFABMS m/z 601.3351 $[M+Na]^+$ (calcd for $C_{32}H_{50}O_9Na$, 601.3353, Δ −0.2 mmu).

2.3.8. Hydrolysis of gagunin A (1). To a stirred solution of **1** (2.0 mg) in 95% EtOH (2 mL) was added KOH (3 mg). After stirring at 40°C for 4 h, the reaction mixture was diluted with water (2 mL), acidified with 0.5N HCl (1 mL), then concentrated under reduced pressure. The organic layer was extracted with $CHCl_3$ (0.5 mL) to yield **8** (0.6 mg) as pure product. Compound **8**: colorless gum; ^{1}H NMR (CD_3OD) δ 5.38 (1H, br d, J=5.3 Hz, H-2), 4.29 (1H, br s, H-11), 4.04 (1H, br s, H-4), 3.94 (1H, br d, J=4.9 Hz, H-12), 3.68 (1H, dd, J=12.2, 4.4 Hz, H-9), 3.66 (1H, d, J=9.8 Hz, H-6), 3.40 (1H, d, J=9.8 Hz, H-5), 3.18 (1H, dd, J=10.8, 5.3 Hz, H-1), 2.14 (1H, dd, J=13.2, 4.4 Hz, H-8α), 1.97 (2H, m, H-13, H-15), 1.82 (1H, br dd, J=10.8, 2.4 Hz, H-14), 1.76 (3H, s, H-18), 1.51 (1H, dd, J=13.2, 12.2 Hz, H-8β), 1.17 (3H, s, H-20), 1.02 (3H, d, J=5.4 Hz, H-16), 0.98 (3H, d, J=7.8 Hz, H-17), 0.80 (3H, s, H-19); ^{13}C NMR (CD_3OD) δ 138.3 (CH, C-2), 134.0 (C, C-3), 83.2 (CH, C-11), 81.7 (CH, C-12), 81.6 (CH, C-6), 76.7 (CH, C-4), 73.2 (CH, C-9), 72.6 (CH, C-5), 54.9 (CH, C-13), 54.6 (CH, C-14), 49.8 (C, C-10), 45.7 (C, C-7), 42.0 (CH_2, C-8), 39.0 (CH, C-1), 24.83 (CH_3, C-18), 24.80 (CH, C-15), 24.3 (CH_3, C-17), 22.6 (CH_3, C-20), 22.4 (CH_3, C-16), 13.3 (CH_3, C-19); LRFABMS m/z 393.2 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_6Na$, 393.2253).

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