Polyoxygenated Diterpenes from the Sponge *Phorbas* sp.

Kyoung Hwa Jang,[†] Ju-eun Jeon,[†] Sungkwang Ryu,[†] Hyi-Seung Lee,[‡] Ki-Bong Oh,*[,§] and Jongheon Shin*[,†]

Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Shilim, Kwanak, Seoul 151-742, Korea, Marine Natural Products Laboratory, Korea Ocean Research & Development Institute, Ansan P.O. Box 29, Seoul 425-600, Korea, and Department of Agricultural Biotechnology, College of Agriculture & Life Science, Seoul National University, San 56-1, Shilim, Kwanak, Seoul 151-921, Korea

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Ten new polyoxygenated diterpenes (**7**−**16**) along with six known gagunin compounds (**1**−**6**) were isolated from the sponge *Phorbas* sp. collected in the Korean Sea. On the basis of a combination of NMR and mass spectroscopic analyses, the molecular structures of these diterpenes, designated as gagunins H−Q, were determined to be penta- or hexa-oxygenated diterpenes of the 10,13-bis-*epi*-homoverrucosane class. A new diterpene acid (**17**) of a bisabolane-related skeletal class was also isolated and structurally defined by the spectroscopic analyses. These compounds exhibited moderate to significant cytotoxicity against the K-562 cell line as well as weak inhibitory activity against isocitrate lyase (ICL).

Sponges have produced numerous biologically active and structurally unique metabolites.[1] Despite their wide geographic distribution in the marine environment, investigations of the thickly encrusting sponges belonging to the genus *Phorbas* (order Poecilosclerida, family Hymedesmiidae) began much later than for other sponge genera. However, since the finding of the phorbazoles, chlorinated phenylpyrrolyloxazoles reported in the mid 1990s, novel metabolites have been continuously isolated from these animals.[2] The most notable examples are the phorboxazoles, highly functionalized macrolides that exhibit potent cytostatic activity.[3−6] Other chemical species isolated from *Phorbas* include the phorbasins, which are essentially diterpenoids with skeletal rearrangements, the phorbasides, which are 14-membered lactone metabolites, and the phorbasterones and amaranzole A, which are steroids bearing unique functional groups.[7−11]

Sponges of the genus *Phorbas* are common in the waters off the southwestern coast of Korea. Our previous studies of these animals yielded gagunins, cytotoxic diterpenoids with highly oxygenated functionalities on a 10,13-*epi*-homoverrucosane skeleton.[12] Similar tricyclic verrucosane diterpenoids and related compounds have been frequently isolated from terrestrial liverworts.[13−18] In the marine environments, this class of compounds have been reported from sponges of the genera *Axinyssa*, *Epipolas*, *Higginsia*, and *Myrmekioderma* as well as from a species of gliding bacterium.[19−25] However, none of these verrucosanes have a high level of oxygenated functionalities, as do those of the gagunins. In our continuing search for bioactive substances from marine organisms of Korea, we re-collected the same *Phorbas* sponge species, the organic extract of which showed considerable toxicity against brine shrimp larvae (LC_{50} 37 ppm). We describe herein the isolation and structure determination of 10 new gagunin compounds that exhibited moderate to significant cytotoxicity against the K-562 cell line and weak inhibition of isocitrate lyase. Additionally, a new diterpene acid related to the bisabolane sesquiterpenes was isolated and structurally defined.

Results and Discussion

The sponge *Phorbas* sp. was collected at 20−25 m off the coast of Gagu-do, Southwest Korea. The lyophilized specimens were repeatedly extracted with MeOH and CH_2Cl_2, respectively. The crude extracts were combined and fractionated employing solvent partitioning. Bioactivity-guided separation of the moderately polar fractions was then accomplished by C_{18} reversed-phase vacuum flash chromatography followed by reversed-phase and silica HPLC to afford compounds **1**−**17**. The structures of the major metabolites **1** and **2** as well as four additional ones, **3**−**6**, were identified to be gagunins A−D, F, and G, respectively, on the basis of combined spectroscopic analyses. The NMR data of these compounds were in good agreement with those reported previously.

The spectroscopic data of gagunin H (**7**, $C_{37}H_{58}O_{11}$) were similar to those of previously reported gagunin terpenes. The ^{13}C NMR spectrum of **7** displayed several signals indicative of ester and oxymethine groups at δ_C ~170 and 82−72 (Table 1). Strong infrared absorption bands at 1735 and 3500 cm^{-1} suggested the presence of ester and hydroxyl groups. The ^1H NMR splitting patterns at lower chemical shifts (δ_H 5.5−3.5) ruled out the possibility of a sugar moiety. The ^{13}C NMR chemical shifts at δ_C 137.3 (CH) and 134.0 (C) indicated a trisubstituted double bond. These spectroscopic data, when considered alongside the nine degrees of unsaturation deduced from the molecular formula, revealed a tricyclic structure for compound **7**.

The assignment of structure **7** to gagunin H was then determined through a combination of ^1H COSY, TOCSY, *g*HSQC, and *g*HMBC NMR experiments. The methyl proton signals of an isopropyl group at δ_H 0.97 and 0.82 were part of a spin system that included oxymethine protons at δ_H 5.43 and 5.04 and an olefinic proton at δ_H 5.46 (Table 1). Proton signals from three oxymethines at δ_H 5.45, 4.95, and 4.14 were coupled in a linear array. An isolated methylene-methine spin system consisting of signals at δ_H 1.65, 1.53, and 4.88 was also observed.

Partial structures were established by detailed interpretation of the gHMBC data (Table 1). Long-range correlations between the upfield methyl protons and neighboring carbon atoms were crucial to the identification of ring junctions and the location of the oxygenated carbon atoms. A methyl singlet at δ_H 1.13 (CH_3-20) exhibited long-range correlations with carbon signals at δ_C 78.9 (CH-11), 74.1 (CH-9), 53.4 (CH-14), and 47.6 (C-10), implying that the former three methine carbon atoms were connected to the latter quaternary carbon. The combination of the ^1H spin systems observed by an analysis of the COSY spectrum with the aforementioned HMBC correlations allowed the construction of an isopropyl-substituted five-membered ring. Long-range correlations were also observed between the vinyl methyl protons at δ_H 1.84 (CH_3-18) and carbon atoms at δ_C 137.3 (CH-2), 134.0 (C-3), and 74.7 (CH-4). Direct proton−proton coupling (J = 5.4 Hz) was observed between methine (δ_H 3.50, δ_C 36.5) and olefinic protons

* To whom correspondence should be addressed. (J.S.) Tel: 82 2 880 2484. Fax: 82 2 762 8322. E-mail: shinj@snu.ac.kr. (K.-B.O.) Tel: 82 2 880 4646. Fax: 82 2 873 3112. E-mail: ohkibong@snu.ac.kr.
[†] College of Pharmacy, Seoul National University.
[‡] Korea Ocean Research & Development Institute.
[§] College of Agriculture & Life Sciences, Seoul National University.

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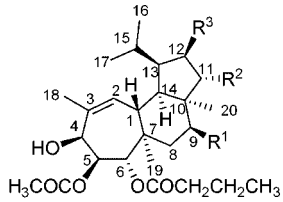
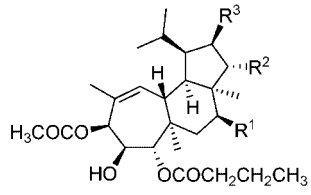
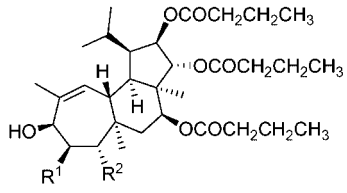
Chart 1



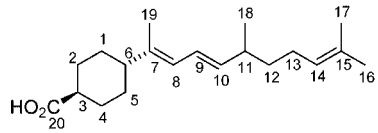
	R^1	R^2	R^3
1	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$	$OCOCH_2CH(CH_3)_2$
2	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$
3	$OCOCH_2CH_2CH_3$	$OCOCH_3$	$OCOCH_2CH_2CH_3$
4	$OCOCH_2CH_2CH_3$	H	$OCOCH_2CH_2CH_3$
7	$OCOCH_2CH_3$	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$
8	$OCOCH_3$	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$
9	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_3$	$OCOCH_2CH_2CH_3$
10	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_2CH_3$
11	$OCOCH_3$	H	$OCOCH_2CH_2CH_3$
12	$OCOCH_2CH(CH_3)_2$	H	$OCOCH_2CH_2CH_3$



	R^1	R^2	R^3
5	$OCOCH_2CH_2CH_3$	H	$OCOCH_2CH_2CH_3$
6	$OCOCH_2CH_2CH_3$	H	$OCOCH_3$
13	$OCOCH_3$	H	$OCOCH_2CH_2CH_3$
14	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$	$OCOCH_2CH_2CH_3$



	R^1	R^2
15	$OCOCH_3$	$OCOCH_3$
16	$OCOCH_2CH_3$	$OCOCH_2CH_2CH_3$



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(δ_H 5.46, δ_C 137.3). These correlations, coupled with the linear array of three oxymethines at δ_H 5.45, 4.95, and 4.14 from COSY data, were indicative of a seven-membered ring at the terminus of the molecule. A six-membered ring at the center of the molecule was identified by long-range correlations of protons at δ_H 4.88 (H-9), 1.65/1.53 (CH_2-8), and 1.03 (CH_3-19) with neighboring carbon atoms. In particular, long-range correlations of the methyl singlet at δ_H 1.03 with carbon atoms at δ_C 78.0 (CH-6), 44.7 (C-7), 36.5 (CH-1), and 36.3 (CH_2-8) were crucial to the assignment of this ring between five- and seven-membered rings. The deduced planar carbon framework of **7** is typical of homoverrucosane-related compounds and is consistent with the known structures of other gagunins.

Gagunin H (**7**) possessed oxygenated groups at C-4, C-5, C-6, C-9, C-11, and C-12. The identification and assignment of these functionalities were accomplished with combined 2-D NMR analyses. The gHMBC data showed long-range correlations between these atoms and H-5 methine and singlet methyl protons at δ_H 2.04, consistent with an acetoxyl group on C-5. Similarly, a propyroxyl group was assigned to C-9 based on gHMBC correlations of the carbonyl carbon at δ_C 174.2 with the H-9 and propyroxyl protons, including the methyl protons at δ_H 1.06. Three butyroxyl groups were revealed by tracing proton couplings with the upfield methyl protons at δ_H 0.97, 0.94, and 0.92 in ^{1}H COSY and TOCSY data. The gHMBC correlations of the three ester carbons with oxymethine protons allowed the assignment of the three butyroxyl groups to C-6, C-11, and C-12. The remaining hydroxyl group was assigned to C-4 on the basis of the chemical shift of the H-4 methine proton at δ_H 4.14. Thus, the planar structure of **7** was determined to be that of a 5-acetoxy-6,11,12-tributyroxy-4-hydroxy-9-propyroxyhomoverrucosa-2-ene.

Stereochemical assignments of asymmetric centers in **7** were based on ROESY and 1-D NOESY experiments (Figure 1). The H-1 proton at the A/B ring junction exhibited a cross-peak correlation with H-6, which in turn correlated with H-8β (δ_H 1.53) (Table 1). Conversely, the CH_3-19 methyl group on the same ring junction showed cross-peak correlations with H-5, H-8α (δ_H 1.65), H-9, and H-14, implying a *trans* A/B ring junction. 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 a 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. Together with the NOE correlations between H-9 and H-20, and H-14 and H-19, these data suggested a *cis* B/C ring junction and α-orientations for H-12, H-13, H-14, and H-20. The H-11 methine proton, uncorrelated with any proton in the C ring, was assigned a β-orientation on the basis of a strong NOE correlation with H-8β. The H-16 and H-17 isopropyl methyl protons exhibited strong NOE correlations with H-12 and H-2, respectively. A three-dimensional molecular model for **7** revealed that steric overcrowding by a bulky substituent at the adjacent C-12 effectively prevents free rotation of the isopropyl group. Restricted rotation would result in the observed differentiation between the H-16 (δ_H 0.82, J = 6.4 Hz) and H-17 (δ_H 0.97, J = 5.9 Hz) methyl groups, consistent with the ^{1}H NMR spectra of **1**−**6**. Thus, the relative configurations of the asymmetric carbon centers of **7** were assigned as 1*R**, 4*S**, 5*S**, 6*S**, 7*R**, 9*S**, 10*R**, 11*R**, 12*R**, 13*R**, and 14*S**.

The NMR spectra of gagunin I (**8**, $C_{36}H_{56}O_{11}$) were very similar to those obtained for **7**. The most noticeable difference in the ^{13}C NMR spectrum was the disappearance of a methylene carbon from the upfield region. Also observed was a significant shift of the propyroxyl methyl carbon from δ_C 9.1 to ~21.2 (Table 2). Corresponding changes were also observed in the ^{1}H NMR spectra; signals at δ_H 1.06 (3 H, t, J = 7.6 Hz) and 2.19 (2 H, m) were replaced by a new signal at δ_H 1.91 (3 H, s) (Table 3). These spectral differences were readily accounted for by replacement of the C-9 propyroxyl group in **7** with an acetoxyl group in **8**. This interpretation was confirmed by combined 2-D NMR data and gHMBC correlations of the acetoxyl carbonyl carbon with H-9.

The molecular formula of gagunin J (**9**, $C_{37}H_{58}O_{11}$) was identical to that of **7**. A combination of ^{1}H COSY, TOCSY, and *g*HSQC experiments showed the same homo- and hetero-NMR correlations in these two compounds. However, detailed examination of the NMR data revealed that several proton and carbon signals originating from the A ring in **9** were noticeably shifted relative to those

Table 1. ^{1}H and ^{13}C NMR Assignments for Compound **7** in $CDCl_3$[a]

position	δ_C	δ_H[b]	HMBC[c]	NOE
1	36.5 CH	3.50, dd (10.8, 5.4)	C-2, C-3, C-6, C-7, C-14, C-19	H-6, H-8β, H-15
2	137.3 CH	5.46, d (5.4)	C-14, C-18	H-14, H-17, H-18
3	134.0 C			
4	74.7 CH	4.14, br s	C-2, C-3, C-5, C-6, C-18	H-5, H-18
5	72.7 CH	4.95, br d (10.1)	C-6, 5-ace(C-1)	H-4, H-19
6	78.0 CH	5.45, d (10.1)	C-1, C-4, C-5, C-7, C-19, 6-bu(C-1)	H-1, H-8β
7	44.7 C			
8	36.3 CH_2	1.65(α), m	C-6, C-7, C-9, C-19	H-9, H-19
		1.53(β), dd (12.8, 12.4)		H-1, H-6, H-11
9	74.1 CH	4.88, dd (12.4, 4.3)	C-11, 9-bu(C-1)	H-8α, H-19, H-20
10	47.6 C			
11	78.9 CH	5.43, br s	C-9, C-10, C-12, 11-bu(C-1), C-20	H-8β
12	81.3 CH	5.04, br d (6.1)	C-10, C-11, C-14, 12-bu(C-1)	H-16
13	52.5 CH	2.17, m	C-14	H-14, H-16, H-17, H-20
14	53.4 CH	2.00, dd (10.5, 5.0)	C-1, C-2, C-12, C-20	H-2, H-13, H-17, H-19, H-20
15	23.8 CH	1.87, m	C-16, C-17	H-1, H-17
16	21.8 CH_3	0.82, d (6.4)	C-13, C-15, C-17	H-12, H-13, H-17
17	23.5 CH_3	0.97, d (5.9)	C-13, C-15, C-16	H-2, H-13, H-14, H-15, H-16
18	24.0 CH_3	1.84, s	C-2, C-3, C-4	H-2, H-4
19	14.2 CH_3	1.03, s	C-1, C-6, C-7, C-8	H-5, H-8α, H-9, H-14
20	22.7 CH_3	1.13, s	C-9, C-10, C-11, C-14	H-9, H-13, H-14
(5-acetoxyl)				
1	170.0 C			
2	21.2 CH_3	2.04, s	5-ace(C-1)	
(6-butyroxyl)				
1	172.4 C			
2	36.2 CH_2	2.22, m	6-bu(C-1, C-3, C-4)	
3	18.5 CH_2	1.60, m	6-bu(C-2)	
4	13.8 CH_3	0.94, t (7.4)	6-bu(C-2,C-3)	
(9-propyroxyl)				
1	174.2 C			
2	27.7 CH_2	2.19, m	9-pr(C-1)	
3	9.1 CH_3	1.06, t (7.6)	9-pr(C-1, C-2)	
(11-butyroxyl)				
1	171.8 C			
2	36.3 CH_2	2.28, m	11-bu(C-3, C-4)	
3	18.4 CH_2	1.68, m	11-bu(C-1)	
4	13.8 CH_3	0.92, t (7.4)	11-bu(C-2, C-3)	
(12-butyroxyl)				
1	172.5 C			
2	36.4 CH_2	2.43, dt (15.1, 7.5)	12-bu(C-1, C-2, C-4)	
		2.34, dt (15.0, 7.5)		
3	18.6 CH_2	1.75, hex (7.3)	12-bu(C-1, C-2)	
4	13.7 CH_3	0.97, t (7.3)	12-bu(C-2, C-3)	

[a] Assignments were aided by ^{1}H COSY, gHSQC, and gHMBC experiments. [b] Multiple means that coupling patterns were not accurately measured due to the overlapping of proton signals. [c] ace, pr, and bu denote acetoxyl, propyroxyl, and butyroxyl, respectively.

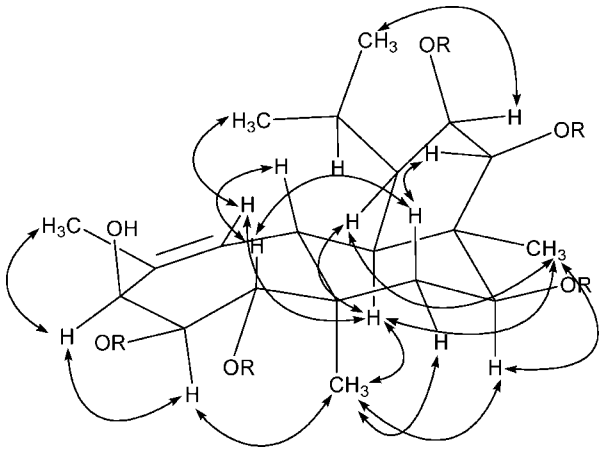

Figure 1. Selected NOE correlations of compound **7**.

in **7**, implying different substituent groups. The gHMBC experiment showed long-range correlations between H-9 and H-11 with the carbonyl carbons at δ_C 173.3 and 171.5, respectively. On the basis of the 2-D NMR data, these carbon atoms were assigned to the carbonyl component of the butyroxyl and propyroxyl groups, respectively. Thus, compound **9** was determined to be a derivative of **7** with the propyroxyl located at C-11.

A related compound, gagunin K (**10**, $C_{39}H_{62}O_{11}$), was isolated as a white solid. The NMR spectra of this compound were very similar to those of **1** and **2**. Closer examination revealed that in addition to an acetoxyl and three butyroxyl groups **10** possessed a linear pentyroxyl group: δ_C 172.7 (C), 34.2 (CH_2), 27.1 (CH_2), 22.4 (CH_2), 13.8 (CH_3), δ_H 2.44 (1 H), 2.36 (1 H), 1.69 (2 H), 1.40 (2 H), 0.97 (3 H) (Tables 2 and 3). This group was assigned to C-12 on the basis of long-range correlations between the carbonyl carbon atom and H-12. Thus, the structure of gagunin K (**10**) was a derivative of gagunin A (**1**) with a pentyroxyl group at C-12.

The molecular structure of gagunin L (**11**, $C_{32}H_{50}O_9$) was similar to that of **8** with the exception of a butyroxyl group. A combination of ^{1}H COSY and TOCSY NMR experiments showed that the H-11 at δ_H 5.42 (1 H, br s) had been replaced by a new methylene proton at δ_H 1.83 (2 H, m). Further evidence was provided by gHMBC data showing long-range correlations between newly added methylene protons and C-9, C-10, C-12, C-13, C-14, and C-20. The corresponding methylene carbon at δ_C 40.5 also displayed long-range correlations with H-9, H-14, and H-20. Thus, the structure of **11** was an 11-desbutyroxyl derivative of **8**.

The spectral characteristics of gagunin M (**12**, $C_{35}H_{56}O_9$) were especially similar to those of compound **11**. The only difference

Table 2. ^{13}C NMR Assignments for Compounds **8**−**16** in $CDCl_3$

position	8	9	10	11	12	13	14	15	16
1	36.3	36.2	36.5	36.2	36.4	37.2	37.4	36.3	36.5
2	137.2	137.3	137.3	138.1	138.1	139.5	138.6	137.2	137.2
3	131.9	131.8	129.9	131.6	131.6	131.2	131.6	131.9	131.9
4	74.4	74.4	74.4	74.5	74.4	78.6	78.4	74.4	74.5
5	72.7	72.6	72.7	72.6	72.6	69.1	69.1	72.6	72.5
6	77.9	78.0	78.0	78.2	78.2	82.3	82.0	78.4	77.9
7	44.7	44.7	44.7	44.8	44.8	44.1	44.1	44.6	44.7
8	36.4	36.4	36.3	36.6	36.6	36.6	36.5	36.4	36.2
9	74.2	74.0	74.0	75.0	74.7	75.0	74.0	73.9	74.0
10	47.5	47.5	47.5	44.6	44.7	44.7	47.9	47.5	47.5
11	78.8	78.9	78.8	40.5	40.5	41.6	79.0	78.8	78.8
12	80.4	80.4	80.5	76.2	76.2	77.9	81.5	80.4	80.5
13	52.5	52.3	52.5	55.0	55.0	55.2	52.3	52.4	52.5
14	53.3	53.3	53.3	52.7	52.7	53.8	54.4	53.4	53.3
15	23.7	23.8	23.7	24.0	23.9	23.9	23.8	23.7	23.7
16	21.7	21.9	21.7	21.9	22.0	21.5	21.3	21.7	21.7
17	23.4	23.5	23.5	23.6	23.7	23.3	23.3	23.4	23.5
18	24.0	24.0	24.0	24.0	24.0	23.8	23.9	24.0	24.0
19	14.1	14.1	14.2	14.1	14.1	15.1	15.0	14.1	14.1
20	22.6	22.6	22.7	29.3	29.2	30.5	23.5	22.6	22.6
(4-acetoxyl)									
1						170.2	170.3		
2						20.9	20.9		
(5-alkyloxyl)									
1	170.0	169.7	170.0	170.7	170.1			170.0	173.6
2	21.1	21.2	21.2	21.2	21.2			20.7	27.7
3									9.0
(6-alkyloxyl)									
1	172.4	172.4	172.4	172.7	172.7	174.4	174.4	169.9	172.4
2	36.2	36.5	36.4	36.5	36.3	36.4	36.3	21.1	36.3
3	18.4	18.4	18.4	18.4	18.4	18.4	18.5		18.4
4	13.7	13.8	13.8	13.8	13.6	13.6	13.7		13.6
(9-alkyloxyl)									
1	170.6	173.3	173.4	170.9	173.5	170.9	173.5	173.4	173.4
2	21.2	36.3	36.5	21.2	43.8	21.3	36.3	36.4	36.3
3		18.4	18.5		36.3		18.4	18.3	18.4
4		13.7	13.7		22.6		13.6	13.5	13.8
5					22.5				
(11-alkyloxyl)									
1	170.7	171.5	170.7				170.6	170.7	170.9
2	36.1	27.6	36.2				36.3	36.2	36.3
3	18.5	9.1	18.5				18.5	18.5	18.4
4	13.8		13.6				13.8	14.1	13.7
(12-alkyloxyl)									
1	172.5	172.6	172.7	173.5	173.0	173.4	172.5	172.5	172.6
2	36.5	36.4	34.2	36.5	36.5	36.5	36.5	36.5	36.4
3	18.6	18.6	27.1	18.7	18.5	18.5	18.6	18.6	18.6
4	13.8	13.6	22.4	13.8	13.8	13.6	13.5	13.7	13.8
5			13.8						

observed was in the NMR data, consisting of the replacement of a 9-acetoxyl group with an isovaleroxyl group: δ_C 173.5 (C), 43.8 (CH_2), 36.3 (CH), 22.6 (CH_3), 22.5 (CH_3), δ_H 2.27 (2 H), 2.20 (1 H), 1.03 (3 H), 1.00 (3 H) (Tables 2 and 3). This interpretation was confirmed by 2-D NMR analysis and comparison of the NMR data with those of **1**, which possesses the same isovaleroxyl group.

Gagunin N (**13**, $C_{32}H_{50}O_9$) was isolated as an amorphous solid. Based on 2-D NMR analysis, the diterpene moiety was identical to those of **11**. However, significant shifts were observed in the ^1H NMR signals of H-4 and H-5, suggesting a change in substituents (Table 4). On the basis of a combination of NMR and gHMBC analyses, these substituents were identified as two acetoxyl, two butyroxyl, and one hydroxyl group, yielding a 4,9-diacetoxy-6,12-dibutyroxy-5-hydroxy derivative. The significant shift of carbon and proton signals associated with ring A are thought to have been derived from the change of an acetoxyl group to a hydroxyl group at C-5 and was consistent with NMR data of the congeners **5** and **6**. This interpretation was also supported by ROESY experiments showing key cross-peaks at H-1/H-15, H-8/H-11, H-12/H-16, H-13/H-17, H-13/H-20, and H-14/H-20.

^1H COSY and TOCSY NMR data revealed that gagunin O (**14**, $C_{38}H_{60}O_{11}$) possessed the same diterpene framework as the other gagunin compounds, but with four butyroxyl, one acetoxyl, and one hydroxyl group. The latter two groups were located on C-4 and C-5, respectively; gHMBC data indicated that the butyroxyl groups were located on C-6, C-9, C-11, and C-12.

NMR analyses of gagunin P (**15**, $C_{36}H_{56}O_{11}$) indicated the same oxidation pattern as those of compounds **7**−**10**, implying that structural differences exist only in the type and location of substituent groups. On the basis of 2-D NMR experiments, these were defined and assigned as 5,6-diacetoxy-9,11,12-tributyroxy-4-hydroxy substituents. A related compound, gagunin Q (**16**, $C_{39}H_{62}O_{11}$), possessed the same diterpene moiety as **15**, but with 6,9,11,12-tetrabutyroxy-4-hydroxy-5-propyroxy substituents.

In addition to gagunin diterpenoids, compound **17** was isolated as a colorless oil with the molecular formula $C_{20}H_{32}O_2$. Although the ^{13}C NMR spectra of this compound showed signals corresponding to only 18 carbon atoms, ^1H NMR integration and gHSQC data indicated 20 carbon atoms. This observation, coupled with the presence of four upfield methyl proton signals in the NMR data, suggested a diterpene structure. However, the NMR chemical shifts and coupling patterns differed significantly from those of the gagunin compounds. For example, a carbonyl carbon at δ_C 181.8 in the ^{13}C NMR spectra, combined with an IR absorption band at 1702 cm^{-1}, indicated the presence of a carboxylic acid. In addition, a UV absorption maximum at 229 nm was indicative of a conjugated diene system.

Proton−proton coupling (modes) of olefinic protons at δ_H 6.22−5.10 in the ^1H COSY spectra revealed a conjugated diene and a trisubstituted double bond (Table 5). The presence of three vinyl methyl substituents at these double bonds was secured by gHMBC data showing long-range proton−carbon couplings between the olefin and methyl groups. A combination of proton COSY and TOCSY experiments revealed a connecting alkyl chain between these double bonds. Similarly, a doublet methyl proton at δ_H 1.01 in the ^1H NMR data was placed at the methine moiety adjacent to the diene based on proton COSY and gHMBC data.

The remaining structure was elucidated by ^1H COSY spectra showing direct couplings between a methine proton at δ_H 2.29 and two two-proton signals at δ_H 2.09 and 1.49. The gHSQC data showed that these two protons were attached to a single methylene carbon at δ_C 29.1 in the ^{13}C NMR spectra. A similar relationship was observed between a methine proton at δ_H 1.90 and two protons at δ_H 1.82 and 1.28 attached to a methylene carbon at δ_C 30.7. These phenomena suggested a symmetric cycloalkane system. The gHMBC data confirmed a 3,6-disubstituted cyclohexane by long-range correlations of these protons with neighboring carbon atoms. Coupling interactions between these carbon atoms and ring protons implied a carboxylic acid and alkyl side chain at C-3 and C-6, respectively. The orientations of H-3 and H-6 were assigned as axial based on coupling constant analyses and NOESY cross-peaks at H-1ax (H-5ax)/H-3 and H-2ax (H-4ax)/H-6. Thus, compound **17** represents a new diterpene carboxylic acid with an extended bisabolane-type sesquiterpene skeleton.

Previous studies performed in our laboratory revealed moderate to significant cytotoxic activity of gagunins A−G against the K-562 cell line. The presence of ester substituents was essential for the cytotoxicity of these compounds.[12] The same set of experiments with the newly isolated gagunins revealed inhibitory activity against K-562 cells, with LC_{50} values of 18.1, 3.5, 0.37, 0.19, 0.25, 0.45, 10.0, 11.5, 9.1, 17.5, 12.5, 0.71, >50, 11.1, 8.5, and >50 μg/mL for compounds **1**−**16**, respectively. These data were consistent with those of previous findings linking bioactivity to the size of the substituent groups at C-11 of the cyclopentane ring. In contrast to their cytotoxic effects, these compounds were inactive against both Gram-positive and -negative bacteria and pathogenic fungi despite a weak inhibition (LC_{50} 55−140 μg/mL) of isocitrate lyase, a key

Table 3. ^{1}H NMR Assignments for Compounds **8**−**12** in $CDCl_3$

position	**8**	**9**	**10**	**11**	**12**
1	3.48, dd (10.8, 5.8)	3.49, dd (9.8, 6.0)	3.50, dd (10.4, 5.8)	3.49, dd (10.0, 5.9)	3.48, dd (9.6, 6.0)
2	5.43, d (5.8)	5.44, d (6.0)	5.45, d (5.8)	5.50, d (5.9)	5.48, d (6.0)
4	4.11, br s	4.12, br s	4.12, br s	4.11, br s	4.13, br s
5	4.94, dd (10.3, 1.5)	4.94, dd (10.2, 1.5)	4.94, br d (10.0)	4.95, dd (10.3, 1.5)	4.96, dd (10.1, 1.4)
6	5.45, d (10.3)	5.45, d (10.2)	5.43, d (10.0)	5.41, d (10.3)	5.41, d (10.1)
8	1.65, m	1.63, dd (12.7, 4.2)	1.64, m	1.55, dd (12.6, 4.3)	1.55, dd (12.5, 4.1)
	1.53, dd (13.0, 12.3)	1.52, dd (12.7, 12.4)	1.52, dd (12.7, 12.4)	1.35, dd (12.6, 12.6)	1.35, dd (12.5, 12.5)
9	4.86, dd (12.3, 4.0)	4.88, dd (12.4, 4.2)	4.88, dd (12.4, 4.1)	4.89, dd (12.6, 4.3)	4.92, dd (12.5, 4.1)
11	5.42, br s	5.43, br s	5.42, br s	1.83, m	1.88, d (4.4)
12	5.02, br d (6.5)	5.04, br d (6.1)	5.03, br d (6.1)	5.13, dt (5.0, 4.5)	5.14, dt (4.7, 4.4)
13	2.17, m	2.14, m	2.20, m	1.95, m	1.96, m
14	1.98, dd (10.0, 5.6)	2.00, dd (10.9, 5.0)	1.99, dd (10.5, 5.0)	1.80, dd (10.3, 4.8)	1.83, dd (10.1, 4.8)
15	1.86, m	1.92, m	1.86, m	1.85, m	1.94, m
16	0.81, d (6.0)	0.81, d (6.4)	0.81, d (6.4)	0.85, d (5.5)	0.86, d (6.0)
17	0.94, d (6.0)	0.97, d (6.1)	0.96, d (5.9)	0.94, d (6.0)	0.95, d (5.7)
18	1.83, s	1.84, s	1.83, s	1.83, s	1.84, s
19	1.00, s	1.02, s	1.02, s	1.00, s	1.01, s
20	1.12, s	1.13,s	1.13, s	1.06, s	1.07, s
(5-acetoxyl)					
2	2.03, s	2.04, s	2.04, s	2.03, s	2.04, s
(6-butyroxyl)					
2	2.21, m	2.25, m	2.32, m	2.28, m	2.26, m
3	1.62, m	1.61, m	1.58, m	1.60, m	1.59, m
4	0.93, t (7.3)	0.89, t (7.3)	0.95, t (7.5)	0.93, t (7.5)	0.92, t (7.3)
(9-alkyloxyl)					
2	1.91, s	2.15, m	2.16, m	1.98, s	2.27, m
3		1.56, m	1.64, m		2.20, m
4		0.93, t (7.4)	0.93, t (7.4)		1.03, d (5.8)
5					1.00, d (5.8)
(11-butyroxyl)					
2	2.17, m	2.29, m	2.20, m		
3	1.66, m	1.02, t (7.6)	1.64, m		
4	0.96, t (7.3)		0.90, t (7.4)		
(12-alkyloxyl)					
2	2.41, dt (15.0, 7.5)	2.42, dt (15.0, 7.4)	2.44, dt (15.2, 7.6)	2.36, m	2.23, m
	2.33, dt (15.0, 7.5)	2.33, dt (15.0, 7.4)	2.36, dt (15.2, 7.6)		
3	1.73, m	1.74, m	1.69, m	1.72, m	1.61, m
4	1.02, t (7.3)	1.03, t (7.4)	1.40, hex (7.3)	0.99, t (7.5)	0.93, t (7.4)
5			0.97, t (7.3)		

enzyme in microbial metabolism. Compound **17** was inactive in all of the above assays.

Experimental Section

General Experimental Procedures. Optical rotation measurements were obtained on a Jasco P-1020 polarimeter with a 1 cm path-length cell. IR and UV absorption spectra were recorded on a Jasco FT/IR 4200 and a Hitachi U-3010 spectrophotometer, respectively. NMR spectra were recorded in $CDCl_3$ solutions containing Me_4Si as an internal standard, on Varian Gemini 2000, Varian Unity 500, and Bruker Avance 600 spectrometers. Proton NMR spectra were measured at 300, 500, and 600 MHz, respectively. High-resolution fast-atom bombardment mass spectrometry (HRFABMS) was performed at the Daegu Branch of the Korea Basic Science Institute on a Jeol JMS 700 high-resolution mass spectrometer. Molecular formulas were determined from ^{13}C NMR and HRFABMS analyses. All solvents were spectral grade or were distilled from glass prior to use.

Animal Material. Specimens of *Phorbas* sp. (sample number 06SH-4) were collected by hand with scuba equipment at a depth of 20−25 m off the shore of Gagu-do, Korea, in July 2006. The surface morphology of the sponges was irregular and composed of mountain-like peaks. Oscules were rare and the texture was very soft. While alive, the samples were dark red and the gross morphological features were very similar to those previously identified and deposited (registry number Spo. 37) at the Natural History Museum, Hannam University, Korea, under the curatorship of C. J. Sim.

Extraction and Isolation. The freshly collected sponge 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 (3 L × 3) and CH_2Cl_2 (3 L × 3). The combined crude extract (1180 g) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was evaporated to dryness in vacuo, and the residue (151.1 g) was partitioned between 15% aqueous MeOH (35.4 g) and *n*-hexane (99.4 g). An aliquot (8.1 g) of the aqueous MeOH layer 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% MeOH(aq), 100% MeOH). On the basis of the result of TLC analysis, the fractions eluted with 10% MeOH(aq) (2.48 g) and 100% MeOH (2.51 g) were chosen for separation.

An aliquot (1.73 g) of the fraction eluted with 10% MeOH(aq) was dried and separated by reversed-phase HPLC (YMC-ODS-A column, 20% MeOH(aq)) to afford, in the order of elution, compounds **13**, **11**, **6**, **3**, **8**, **5**, **4**, **15**, **9**, **7**, **12**, **14**, **2**, and **1** as white solids. An aliquot (1.75 g) of the fraction eluted with 100% MeOH was dried and separated by reversed-phase HPLC (YMC-ODS-A column, 15% MeOH(aq)) to afford, in the order of elution, compounds **4**, **12**, **2**, **1**, **16**, **10**, and **17** as white solids. Proton NMR analysis revealed that some compounds were mixtures of two or more and the others contained impurities. Purification of these metabolites was then accomplished by normal-phase HPLC (YMC-silica column, 25% EtOAc in *n*-hexane for compounds **1**−**9** and **11**−**15**, 20% EtOAc in *n*-hexane for compounds **4**, **10**, **12**, **16**, and **17** in another fraction.) The purified metabolites were isolated in the following amounts: 100.6, 238.5, 38.2, 64.4, 15.1, 7.2, 5.0, 4.0, 7.7, 3.8, 14.8, 13.1, 6.6, 4.3, 7.6, 11.7, and 4.5 mg for **1**−**17**, respectively.

Gagunin A (1): amorphous solid; [α] +48.6 (*c* 0.90, MeOH); IR (ZnSe) ν_{max} 3450 (br), 2965, 1735, 1460, 1375, 1245 cm^{-1}; HRFABMS *m*/*z* 729.4188 [M + Na]$^+$ (calcd for $C_{39}H_{62}O_{11}Na$, 729.4190).

Gagunin B (2): amorphous solid; [α] +55.3 (*c* 0.75, MeOH); IR (ZnSe) ν_{max} 3450 (br), 2965, 1735, 1460, 1375, 1245 cm^{-1}; HRFABMS *m*/*z* 693.4215 [M + H]$^+$ (calcd for $C_{38}H_{61}O_{11}$, 693.4214).

Gagunin C (3): amorphous solid; [α] +54.1 (*c* 1.5, MeOH); IR (ZnSe) ν_{max} 3450 (br), 2960, 1735, 1460, 1370, 1240 cm^{-1}; HRFABMS *m*/*z* 665.3920 [M + H]$^+$ (calcd for $C_{36}H_{57}O_{11}$, 665.3901).

Table 4. ^{1}H NMR Assignments for Compounds **13**−**16** in $CDCl_3$

position	**13**	**14**	**15**	**16**
1	3.16, dd (7.7, 6.0)	3.05, dd (7.8, 6.0)	3.48, dd (10.8, 5.3)	3.50, dd (10.4, 6.0)
2	5.33, d (6.0)	5.32, d (6.0)	5.43, d (5.3)	5.44, d (6.0)
4	5.05, br s	5.06, br s	4.11, br s	4.12, br s
5	3.74, br d (9.5)	3.73, br d (9.5)	4.93, dd (10.2, 1.5)	4.96, dd (10.0, 1.2)
6	5.12, d (9.5)	5.14, d (9.5)	5.42, d (10.2)	5.46, d (10.0)
8	1.61, dd (12.4, 3.3)	1.72, dd (12.6, 3.5)	1.65, m	1.64, m
	1.26, m	1.42, dd (12.6, 12.6)	1.53, dd (12.6, 12.4)	1.52, dd (12.6, 12.4)
9	4.93, dd (12.4, 3.3)	4.92, dd (12.6, 3.5)	4.89, dd (12.4, 4.5)	4.88, dd (12.4, 4.2)
11	1.78, m	5.38, br s	5.43, br s	5.43, br s
12	5.17, dd (5.6, 5.6)	5.07, br d (6.9)	5.02, br d (6.5)	5.03, br d (6.1)
13	1.91, m	2.13, m	2.17, m	2.18, m
14	1.82, m	2.01, dd (10.0, 5.0)	1.98, dd (10.0, 5.0)	2.00, dd (10.6, 5.0)
15	1.64, m	1.59, m	1.86, m	1.85, m
16	0.87, d (7.4)	0.85, d (6.5)	0.81, d (6.0)	0.82, d (6.4)
17	0.88, d (7.4)	0.92, d (6.6)	0.96, d (6.0)	0.97, d (5.5)
18	1.91, s	1.91, s	1.83, s	1.84, s
19	1.01, s	1.02, s	1.01, s	0.97, s
20	1.09, s	1.12, s	1.11, s	1.13, s
(4-acetoxyl)				
2	2.14, s	2.14, s		
(5-alkyloxyl)				
2			2.04, s	2.28, m
3				1.12, t (7.1)
(6-alkyloxyl)				
2	2.35, t (7.3)	2.35, m	1.99, s	2.34, m
3	1.68, m	1.66, m		1.62, m
4	0.95, t (7.4)	0.95, t (7.1)		0.87, t (7.4)
(9- alkyloxyl)				
2	1.99, s	2.18, m	2.11, dt (15.0, 7.5)	2.17, m
			2.17, dt (15.0, 7.5)	
3		1.57, m	1.56, m	1.68, m
4		0.90, t (7.7)	0.89, t (7.5)	1.02, t (7.4)
(11-butyroxyl)				
2		2.30, m	2.24, m	2.25, m
3		1.66, m	1.67, m	1.67, m
4		0.97, t (7.0)	1.02, t (7.3)	1.01, t (7.4)
(12-butyroxyl)				
2	2.53, dt (15.8, 7.5)	2.53, dt (15.6, 7.4)	2.42, dt (15.0, 7.4)	2.42, dt (15.0, 7.5)
	2.38, dt (15.8, 7.5)	2.37, dt (15.6, 7.4)	2.32, dt (15.0, 7.4)	2.33, dt (15.0, 7.5)
3	1.78, m	1.78, m	1.74, hex (7.3)	1.75, p (7.4)
4	1.02, t (7.4)	1.04, t (7.2)	0.97, t (7.6)	0.92, t (7.4)

Table 5. NMR Assignments for Compounds **17** in $CDCl_3$

position	δ_C	δ_H	HMBC
1	30.7 CH_2	1.82, br d (13.3);	C-2, C-3, C-5, C-6
		1.28, br dt (12.8, 13.3)	
2	29.1 CH_2	2.09, br d (12.8);	C-1, C-3, C-4, C-6
		1.49, ddt (12.3, 3.4, 12.8)	
3	43.0 CH	2.29, tt (12.3, 3.5)	C-2, C-4, C-20
4	29.1 CH_2	2.09, br d (12.8);	C-2, C-3, C-5, C-6
		1.49, ddt (12.3, 3.4, 12.8)	
5	30.7 CH_2	1.82, br d (13.3);	C-1, C-3, C-4, C-6
		1.28, br dt (12.8, 13.3)	
6	46.5 CH	1.90, tt (13.3, 3.4)	C-1, C-5, C-7, C-8, C-19
7	140.4 C		
8	123.6 CH	5.81, d (10.7)	C-6, C-9, C-10, C-19
9	125.1 CH	6.22, dd (15.0, 10.7)	C-7, C-8, C-11
10	139.2 CH	5.49, dd (15.0, 7.9)	C-8, C-11, C-18
11	36.9 CH	2.18, dq (7.9,6.7)	C-9, C-10, C-12, C-13, C-18
12	37.4 CH_2	1.34, m	C-10, C-11, C-13, C-14, C-18
13	26.1 CH_2	1.96, dt (7.1, 7.4)	C-11, C-12, C-14, C-15
14	124.9 CH	5.10, t (7.1)	C-12, C-13, C-16, C-17
15	131.5 C		
16	26.0 CH_3	1.69, s	C-14, C-15, C-17
17	17.9 CH_3	1.60, s	C-14, C-15, C-16
18	20.9 CH_3	1.01, d (6.7)	C-10, C-11, C-12
19	15.2 CH_3	1.72, br s	C-6, C-7, C-8
20	181.8 C		

Gagunin D (4): amorphous solid; [α] +51.3 (*c* 0.35, MeOH); IR (ZnSe) ν_{max} 3450 (br), 2965, 1730, 1460, 1240 cm^{-1}; HRFABMS *m/z* 607.3840 $[M + H]^+$ (calcd for $C_{34}H_{55}O_9$, 607.3846).

Gagunin F (5): amorphous solid; [α] +157.3 (*c* 0.8, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2965, 1730, 1460, 1380, 1235 cm^{-1}; HRFABMS *m/z* 607.3846 $[M + H]^+$ (calcd for $C_{34}H_{55}O_9$, 607.3846).

Gagunin G (6): amorphous solid; [α] +74.9 (*c* 0.85, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2960, 1735, 1455, 1380, 1240 cm^{-1}; HRFABMS *m/z* 579.3536 $[M + H]^+$ (calcd for $C_{32}H_{51}O_9$, 579.3533).

Gagunin H (7): amorphous solid; [α] +73.2 (*c* 0.75, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2964, 1735, 1456, 1371, 1239 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 679.4059 $[M + H]^+$ (calcd for $C_{37}H_{59}O_{11}$, 679.4057).

Gagunin I (8): amorphous solid; [α] +46.9 (*c* 0.65, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2965, 1732, 1457, 1368, 1243 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRFABMS *m/z* 665.3903 $[M + H]^+$ (calcd for $C_{36}H_{57}O_{11}$, 665.3901).

Gagunin J (9): amorphous solid; [α] +67.5 (*c* 0.80, MeOH); IR (ZnSe) ν_{max} 3502 (br), 2966, 1737, 1459, 1368, 1243 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Tables 2 and 3; HRFABMS *m/z* 679.4066 $[M + H]^+$ (calcd for $C_{37}H_{59}O_{11}$, 679.4057).

Gagunin K (10): amorphous solid; [α] +60.4 (*c* 0.55, MeOH); IR (ZnSe) ν_{max} 3450 (br), 2960, 1735, 1465, 1370, 1240 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRFABMS *m/z* 729.4196 $[M + Na]^+$ (calcd for $C_{39}H_{62}O_{11}Na$, 729.4190).

Gagunin L (11): amorphous solid; [α] +116.4 (*c* 0.50, MeOH); IR (ZnSe) ν_{max} 3450 (br), 2965, 1735, 1460, 1375 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HRFABMS *m/z* 579.3521 $[M + H]^+$ (calcd for $C_{32}H_{51}O_9$, 579.3533).

Gagunin M (12): amorphous solid; [α] +73.6 (*c* 0.85, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2966, 1737, 1459, 1368, 1243 cm^{-1};^1H NMR and ^{13}C NMR data, see Tables 2 and 3; HRFABMS *m/z* 621.4008 $[M + H]^+$ (calcd for $C_{35}H_{57}O_9$, 621.4003).

Gagunin N (13): amorphous solid; [α] +171.0 (*c* 0.60, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2964, 1737, 1457, 1370, 1247 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; HRFABMS *m/z* 579.3536 $[M + H]^+$ (calcd for $C_{32}H_{51}O_9$, 579.3533).

Gagunin O (14): amorphous solid; [α] +146.3 (*c* 0.55, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2960, 1735, 1455, 1380, 1240 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; HRFABMS *m/z* 693.4213 $[M + H]^+$ (calcd for $C_{38}H_{61}O_{11}$, 693.4214).

Gagunin P (15): amorphous solid; [α] +55.1 (*c* 0.80, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2964, 1735, 1456, 1371, 1239 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; HRFABMS *m/z* 665.3903 $[M + H]^+$ (calcd for $C_{36}H_{57}O_{11}$, 665.3901).

Gagunin Q (16): amorphous solid; [α] +68.3 (*c* 0.80, MeOH); IR (ZnSe) ν_{max} 3500 (br), 2960, 1735, 1455, 1380, 1240 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 4; HRFABMS *m/z* 707.4366 $[M + H]^+$ (calcd for $C_{39}H_{63}O_{11}$, 707.4370).

Compound 17: amorphous solid; [α] +12.2 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.01), 229 (sh, 3.61) nm; IR (ZnSe) ν_{max} 3400 (br), 2936, 1702, 1541, 1257 cm^{-1}; ^1H and ^{13}C NMR data, see Table 5; HRFABMS *m/z* 303.2314 $[M - H]^-$ (calcd for $C_{20}H_{31}O_2$, 303.2324).

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