Euplexides A−E: Novel Farnesylhydroquinone Glycosides from the Gorgonian *Euplexaura anastomosans*

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Euplexides A−E (**1**−**5**), novel farnesylhydroquinone glycosides of the moritoside class, have been isolated from the gorgonian *Euplexaura anastomosans*. The structures of these compounds have been elucidated by combined chemical and spectral methods. The absolute stereochemistry has been determined by the modified Mosher's method and an acidic hydrolysis followed by GC analysis. These compounds exhibited moderate cytotoxicity and antioxidizing activity as well as inhibitory activity against PLA_2.

Introduction

Gorgonians (sea whips and sea fans; phylum Cnidaria, order Gorgonacea) have produced a wide variety of structurally unique and biologically active secondary metabolites; terpenoids and steroids of uncommon carbon skletons and functionalities are the major groups of metabolites.[1−3] Gorgonian-derived terpenoids are particularly well recognized for their diverse carbon skeletons and potent bioactivities that make these compounds as attractive targets for both biomedical and synthetic studies.[1,3] As a part of our search for bioactive substances from marine organisms, we recently reported the structures of anastomosacetals A−D, novel steroidal hemiacetals of the cholestane class from the gorgonian *Euplexaura anastomosans* Brundin (Family Plexauridae) collected from Keomun Island, Korea.[4] Both of the organic crude extract from the specimens and the silica flash chromatographic fractions containing anastomosacetals exhibited considerable cytotoxicity (LC_{50} 73.3 and 25.1 μg/mL against HeLa cell line for the extract and chromatographic fractions, respectively) and brine-shrimp lethality (LC_{50} 303 and 95 ppm for the former and the latter, respectively). However, the same measurements using pure compounds showed that anastomosacetals were not active (LC_{50} > 100 μg/mL and >1000 ppm for each test). Careful reexamination of the ^{1}H NMR spectra of chromatographic fractions revealed the presence of several metabolites of different structural class as very minor constituents in the chromatographic fractions containing anastomosacetals. Consequently, the chemistry of this animal has been reinvestigated through a large-scale collection followed by selection of chemically homogeneous colonies (chemotype) based on systematic TLC analyses of the extracts from each individual colony.[5]

In this paper we report the structure elucidations and bioactivities of euplexides A−E (**1**−**5**) (Chart 1), novel farnesylhydroquinone glycosides of a rare structural class. All of the compounds are structurally related to moritoside (**6**) previously isolated from a Japanese gorgonian of the genus *Euplexaura*.[6] However, the structures of these compounds differ from each other in that euplexides possessed a β-D-galactose moiety instead of a β-D-altrose of moritoside. In addition, the absolute stereochemistry of an asymmetric carbon center at the farnesyl chain, unassigned for moritoside, was determined by the modified Mosher's method.[7] Euplexides exhibited moderate cytotoxicity and antioxidizing activity as well as inhibitory activity against PLA_2.

Results and Discussion

The chemically homogeneous specimens were freeze-dried, macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined crude extracts were subjected to silica vacuum flash chromatography using sequential mixtures of hexane and EtOAc as eluents. Fractions eluted with moderately polar solvents (40−45% EtOAc in hexane) were combined and separated by semi-preparative silica and reversed-phase HPLC to yield pure euplexides.

Euplexide A (**1**) was isolated as an amorphous solid that analyzed for $C_{34}H_{48}O_{11}$ by a combination of HR-FABMS and ^{13}C NMR analysis. The presence of an

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(7) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.

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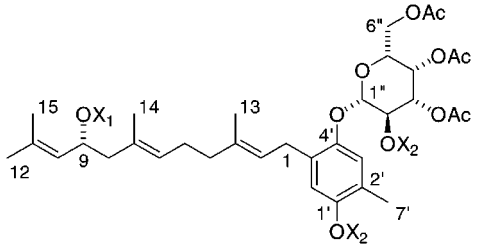
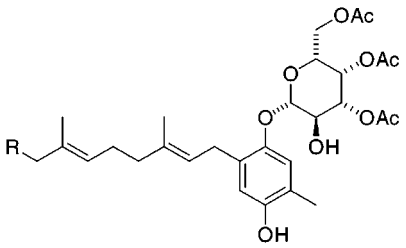
Chart 1



1 : $X_1 = X_2 =$ H
1S : $X_1 = X_2 =$ (*S*)-MTPA
1R : $X_1 = X_2 =$ (*R*)-MTPA
2 : $X_1 =$ Ac, $X_2 =$ H



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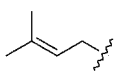

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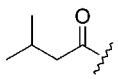

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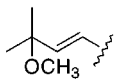
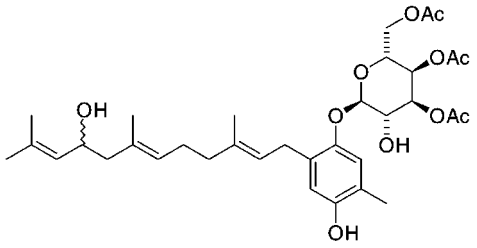




6, moritoside

aromatic ring was revealed by an occurrence of several carbon signals in the region of δ 150−115 in the ^{13}C NMR spectrum and proton signals at δ 6.90 (1H, br s) and 6.50 (1H, s) in the ^{1}H NMR spectrum (Tables 1 and 2). This interpretation was supported by an absorption maximum at 285 (log ϵ 3.49) nm in the UV spectrum. Similarly, the presence of a sugar moiety was readily recognized by characteristic carbon signals in the region of δ 100−60 in the ^{13}C NMR spectrum. Several of the corresponding proton signals were observed at δ 5.5−5.0 in the ^{1}H NMR spectrum that was significantly shifted downfield compared to those of ordinary sugar protons. Since the NMR and IR data indicated the presence of three acetoxyl groups, the downfield shift of the sugar protons appeared to be due to the attachment of these groups to the sugar moiety.

With the aid of this information, the structure of **1** was determined by a combination of ^{1}H COSY, TOCSY, HSQC, and HMBC experiments. The HMBC data were particularly helpful to determine both the partial structures and connectivities among them. The presence of three trisubstituted double bonds was indicated by the

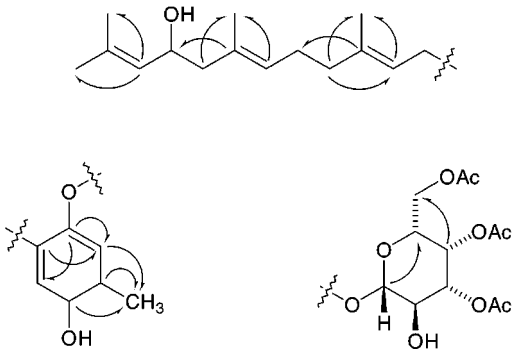


Figure 1. Partial structures and key HMBC (C ⇀ H) correlations of **1**.

^{1}H COSY correlations of the olefinic proton signals with those of vinyl methyl protons. Proton−proton couplings between the signals of olefinic and upfield protons, combined with the HMBC correlations of the vinyl methyl protons with neighboring carbons, defined a farnesyl chain bearing a hydroxyl group at C-9. This interpretation was confirmed by the TOCSY data in which three isolated spin systems containing the olefinic and vinyl methyl protons were clearly observed. The geometry of double bonds at C-2 and C-6 was assigned as *E* for both on the basis of the upfield shifts of the methyl carbon signals in the ^{13}C NMR spectrum, δ 16.2 and 15.5 for C-13 and C-14, respectively.

A combined 2-D NMR analysis of the remaining downfield signals in both ^{1}H and ^{13}C NMR spectra revealed that the aromatic ring was a 1,4-hydroquinone, and the absence of coupling between the signals of aromatic protons at δ 6.90 and 6.50 indicated a para substitution. A long-range coupling of the former with the signal of methyl protons at δ 2.19 (3H, br s) assigned the location of a benzylic methyl group at C-2′. This interpretation was supported by the long-range correlations of the methyl protons with the C-1′, C-2′, and C-3′ carbons. Thus, the structure of the aromatic functionality was defined as a 5-alkyl-2-methyl-1,4-hydroquinone.

The structure of the sugar moiety was also elucidated by combined 2-D NMR experiments. The attachment of three acetyl groups at C-3″, C-4″, and C-6″ carbons was readily determined by chemical shifts of the H-3″, H-4″, and H-6″ protons and also by HMBC correlations between these protons and the acetyl carbonyl carbons. A long-range correlation of the C-1″ acetal carbon at δ 103.9 with the H-5″ proton at δ 3.98 (1H, ddd, J = 7.3, 6.4, and 1.0 Hz) revealed that the sugar was a pyranose. On the basis of the vicinal proton−proton coupling constants, the orientations of the H-1″−H-4″ protons were assigned as axial, axial, axial, and equatorial, respectively. Since the orientation of the H-5″ proton displaying a small vicinal coupling ($J_{4'',5''}$ = 1.0 Hz) was assigned as axial by the mutual NOESY correlations among H-1″, H-3″, and H-5″ protons, the sugar moiety was defined as a β-galactopyranose. Thus, the partial structures of euplexide A were determined as depicted in Figure 1.

Connectivities of the partial structures were also established by HMBC experiments. Long-range correlations of the H-1 methylene protons with the C-4′, C-5′, and C-6′ carbons and also that of the H-6′ proton with the C-1 carbon placed the attachment of the farnesyl chain at C-5′ of the hydroquinone. Similarly the connection between the hydroquinone and galactose by a

Table 1. Proton NMR Assignments for Euplexides A−E (1−5)[a]

no.	1	2	3	4	5
1	3.36, dd (16.1, 8.3)	3.42, dd (16.1, 7.8)	3.42, dd (16.1, 7.3)	3.37, dd (16.6, 7.8)	3.42, dd (16.1, 7.3)
	3.31, dd (16.1, 7.8)	3.25, dd (16.1, 6.4)	3.21, dd (16.1, 6.4)	3.31, dd (16.6, 6.8)	3.23, dd (16.1, 6.4)
2	5.27, dd (8.3, 7.8)	5.25, br dd (7.8, 6.4)	5.25, br dd (7.3, 6.4)	5.25, ddq (7.8, 6.8, 1.5)	5.22, br dd (7.3, 6.4)
4	2.15, m	2.07, m	2.05, m	2.13, m	2.07, m
5	2.30, m; 2.17, m	2.11, m	2.11, m	2.19, m	2.13, m
6	5.26, m	5.18, br t (6.6)	5.10, tq (6.8, 1.0)	5.18, tq (6.8, 1.5)	5.11, ddq (7.3, 6.8, 1.0)
8	2.13, dd (11.2, 7.8)	2.34, dd (13.7, 7.8)	1.99, t (7.3)	3.05, s	2.67, d (6.8)
	2.09, dd (11.2, 2.9)	2.11, m			
9	4.44, ddd (8.3, 7.8, 2.9)	5.60, ddd (9.3, 7.8, 7.8)	2.05, m		5.55, dt (16.1, 6.8)
10	5.19, dhep (8.3, 1.5)	5.13, br dq (7.8, 1.0)	5.09, thep (6.8, 1.5)	2.35, d (7.3)	5.41, d (16.1)
11				2.13, m	
12	1.74, d (1.5)	1.71, s	1.68, d (1.0)	0.90, d (6.4)	1.28, s
13	1.66, br s	1.67, s	1.71, br s	1.64, br s	1.69, br s
14	1.66, br s	1.61, s	1.59, s	1.66, br s	1.57, br s
15	1.69, d (1.5)	1.68, s	1.59, s	0.90, d (6.4)	1.28, s
3′	6.90, br s	6.88, br s	6.87, br s	6.87, br s	6.88, br s
6′	6.50, s	6.60, s	6.57, s	6.59, s	6.57, s
7′	2.19, br s	2.21, br s	2.20, br s	2.21, br s	2.20, br s
1″	4.72, d (7.8)	4.76, d (7.8)	4.75, d (7.8)	4.76, d (7.8)	4.75, d (7.8)
2″	4.05, dd (10.3, 7.8)	4.07, dd (10.3, 7.8)	4.05, dd (10.3, 7.8)	4.07, dd (10.3, 7.8)	4.05, dd (10.3, 7.8)
3″	4.99, dd (10.3, 3.4)	5.00, dd (10.3, 3.4)	4.99, dd (10.3, 3.4)	5.00, dd (10.3, 3.4)	4.99, dd (10.3, 3.4)
4″	5.43, dd (3.4, 1.0)	5.43, br d (3.4)	5.43, dd (3.4, 1.0)	5.42, dd (3.4, 1.0)	5.43, br d (3.4)
5″	3.98, ddd (7.3, 6.4, 1.0)	4.00, br dd (7.3, 6.1)	4.01, ddd (7.3, 5.9, 1.0)	3.99, ddd (6.8, 6.4, 1.0)	4.00, br dd (7.1, 6.4)
6″	4.21, dd (11.2, 7.3)	4.21, dd (11.2, 7.3)	4.21, dd (11.2, 7.3)	4.20, dd (11.2, 6.8)	4.21, dd (11.2, 7.1)
	4.15, dd (11.2, 6.4)	4.15, dd (11.2, 6.1)	4.15, dd (11.2, 5.9)	4.15, dd (11.2, 6.4)	4.15, dd (11.2, 6.4)
OAc	2.16, s	2.16, s	2.16, s	2.16, s	2.16, s
	2.07, s	2.07, s	2.07, s	2.07, s	2.07, s
	2.06, s	2.06, s	2.06, s	2.05, s	2.06, s
9-OAc		2.01, s			
OMe					3.18, s

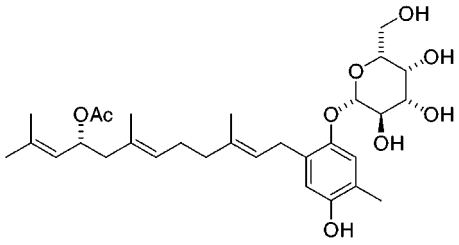
[a] Measured in $CDCl_3$ solutions at 500 MHz; δ in ppm (J in Hz); TMS as internal standard. Assignments were aided by ^{1}H COSY and TOCSY experiments.

glycoside linkage was determined by a long-range correlation between the H-1″ proton and the C-4′ carbon. Thus, the structure of euplexide A was determined as a farnesylhydroquinone β-galactoside.

The farnesyl part of **1** possessed an asymmetric carbon center at C-9. The absolute stereochemistry of this center was determined by the modified Mosher's method.[7] Treatment of **1** with (−)- and (+)-MTPA chloride in pyridine yielded the corresponding triesters, **1***S* and **1***R*, respectively. All of the signals of protons of these compounds were confidently assigned by a combination of ^{1}H COSY and TOCSY experiments. On the basis of the $\Delta(\delta\mathbf{1}S-\delta\mathbf{1}R)$ values around the asymmetric carbon center, the *R* configuration was assigned at C-9 of **1**.

The absolute stereochemistry of the galactose unit was also approached by the Mosher's method. The $\Delta(\delta\mathbf{1}S-\delta\mathbf{1}R)$ values for the H-1″−H-4″ protons suggested that the configuration at C-2″ was *S*, hence a D-galactose. However, the stereochemical assignment based on this method was not conclusive since the $\Delta\delta$ values for the H-5″ (+36 Hz) and H-6″ (+19 and + 3 Hz) protons were much larger than the expected ones (∼0 Hz) from the theory. A three-dimensional model study revealed that the phenyl group of **1***S* (the methoxy group of **1***R*) was very proximal to the acetoxyl group at C-2″. To prevent the spatial crowding, the MTPA group was tilted toward the C-1″ carbon, and thus extension of the MTPA plane reached a point between the C-4″ and C-5″ carbons,[8] hence, the H-5″ and H-6″ protons located at the left side of the plane that would be the reason for the significant positive values of $\Delta\delta$ for both protons.

The absolute stereochemistry of the galactose unit was confirmed by an acidic hydrolysis followed by a GC analysis of the sugar moiety. Acetylation of **1** with acetic anhydride in dry pyridine followed by deacetylation with methanolic ammonia yielded 9-acetyl-3″,4″,6″-trideacetyleuplexide A (**7**).[9] Chiral GC analysis of the acidic hydrolysate of this compound revealed that the sugar moiety was a D-galactose.



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The stereochemistry at the C-9 asymmetric center of moritoside (**6**) was undetermined. A comparison of the optical rotations between euplexide A and moritoside allowed us to infer the absolute configuration of the latter at this center. By use of Hudson's rules of isorotation, the molecular rotation [Φ] was defined as sum of the $[\Phi]_{\text{farnesylhydroquinone}} + [\Phi]_{\text{sugar}}$.[10,11] From the measured optical rotations of **1** and D-galactose, their molecular rotations were calculated as −15.8 and +144.0°, respectively. Therefore, the molecular rotation of the farnesylhydro-

(8) The Mosher's method had limited application for the sterically hindered secondary alcohols. See: (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296−1298. (b) Shin, J.; Seo, Y.; Rho, J.-R.; Baek, E.; Kwon, B.-M.; Bok, S.-H.; Jung, T.-S. *J. Org. Chem.* **1995**, *60*, 7582−7588.

(9) Due to the unstability in a basic condition, **1** could not be directly deacetylated with methanolic ammonia. However, the 9,2″-diacetylated derivative of **1** was stable in the same condition and was deacetylated to yield 9-acetyl-3″,4″,6″-trideacetylated euplexide A.

(10) Lemieux, R. U.; Koto, S. *Tetrahedron* **1974**, *30*, 1933−1944.

(11) Shin, J.; Fenical, W. *J. Org. Chem.* **1991**, *56*, 3153−3158.

Table 2. Carbon NMR Assignments (δ) for Euplexides A−E (1−5)[a]

C no.	1	2	3	4	5
1	27.9 t	28.1 t	28.5 t	27.6 t	28.2 t
2	123.5 d	122.9 d	122.7 d	122.7 d	123.0 d
3	135.6 s	136.3 s	136.9 s	136.0 s	136.3 s
4	39.1 t	39.2 t	39.7 t	38.9 t	39.3 t
5	25.3 t	25.9 t	26.5 t	25.5 t	26.0 t
6	128.8 d	127.2 d	124.0 d	128.5 d	125.0 d
7	131.8 s	130.8 s	135.2 s	128.6 s	133.9 s
8	48.1 t	44.9 t	39.6 t	53.3 t	42.3 t
9	65.6 d	71.0 d	26.7 t	211.8 s	128.6 d
10	126.4 d	123.6 d	124.3 d	51.4 t	136.1 d
11	135.2 s	137.1 s	131.4 s	24.7 d	75.3 s
12	25.8 q	25.7 q	25.7 q	22.5 q	26.0 q
13	16.2 q	16.1 q	16.2 q	15.9 q	16.1 q
14	15.5 q	16.8 q	16.0 q	17.4 q	16.2 q
15	18.2 q	18.5 q	17.7 q	22.5 q	26.0 q
1′	150.4 s	150.1 s	149.6 s	150.6 s	150.0 s
2′	122.4 s	122.2 s	121.9 s	122.2 s	122.2 s
3′	120.0 d	119.5 d	119.2 d	119.7 d	119.4 d
4′	148.4 s	148.4 s	148.8 s	148.1 s	148.6 s
5′	130.1 s	130.2 s	130.4 s	130.1 s	130.3 s
6′	115.3 d	115.8 d	116.1 d	115.2 d	115.9 d
7′	15.8 q	15.9 q	15.8 q	15.8 q	15.8 q
1″	103.9 d	103.4 d	103.2 d	103.5 d	103.4 d
2″	68.9 d	69.1 d	69.1 d	69.2 d	69.1 d
3″	72.6 d	72.5 d	72.4 d	72.5 d	72.5 d
4″	67.3 d	67.2 d	67.1 d	67.2 d	67.2 d
5″	70.9 d	71.0 d	71.0 d	70.9 d	71.0 d
6″	61.7 t	61.7 t	61.7 t	61.6 t	61.7 t
OAc	170.4 s	170.3 s	170.4 s	170.4 s	170.4 s
	170.3 s	170.3 s	170.3 s	170.3 s	170.3 s
	170.2 s	170.1 s	170.2 s	170.2 s	170.2 s
	20.8 q	20.8 q	20.8 q	20.7 q	20.8 q
	20.7 q	20.7 q	20.7 q	20.7 q	20.7 q
	20.7 q	20.7 q	20.6 q	20.6 q	20.6 q
OAc(C-9)		171.0 s			
		21.4 q			
OMe					50.2 q

[a] Measured in $CDCl_3$ at 125 MHz. Assignments were aided by DEPT, HSQC, and HMBC experiments.

quinone of **1** was expected to be −159.8°. Similarly, the molecular rotation of the farnesylhydroquinone of **6** was expected to be +83.4° from the reported optical rotations of **6** and D-altrose. Although there was substantial difference between the calculated molecular rotations of farnesylhydroquinones of **1** and **6**, the opposite signs seem to indicate the 9*S* configuration for the latter. Thus, euplexide A and moritoside defined possess an opposite stereochemistry at the same asymmetric center of the farnesylhydroquinone moiety. However, the stereochemistry of **6** remains to be confirmed by direct spectroscopic analysis.

The molecular formula of euplexide B (**2**), a white solid, was deduced as $C_{36}H_{50}O_{12}$ by combined HRFABMS and ^{13}C NMR analysis. Spectral data for this compound were very similar to those obtained for **1**, with the appearance of NMR signals corresponding to an additional acetoxyl group as the only significant difference, ^1H δ 2.01 (3H, s) and ^{13}C δ 171.0 (C) and 21.4 (CH_3). A combination of 2-D NMR experiments revealed that the new acetoxyl group replaced the C-9 hydroxyl group. Thus, the structure of **2** was determined as the 9-acetyl derivative of **1**.

Another related metabolite, euplexide C (**3**), was isolated as a colorless gum. The molecular formula $C_{34}H_{48}O_{10}$ was established by HRFABMS and ^{13}C NMR analysis. Comparison of the NMR data with those obtained for **1** revealed that the only significant differences were the replacement of signals of the C-9 oxymethine by those of an upfield methylene in both ^1H and ^{13}C NMR spectra. This interpretation was confirmed by a combination of 2-D NMR experiments. Thus, the structure of euplexide C was determined as the 9-dehydroxy derivative of euplexide A.

The molecular formula of euplexide D (**4**), a colorless gum, was established as $C_{34}H_{48}O_{11}$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. The NMR data for this compound were reminiscent of those obtained for **1**. However, the ^{13}C NMR data revealed that the signals of a double bond and C-9 oxymethine were replaced by those of a carbonyl at δ 211.8, a methine at δ 24.7, and a methylene at δ 51.7 (or 53.3). Corresponding differences were also observed in the ^1H NMR spectrum in which signals of protons at δ 4.44 (1H, ddd, J = 8.3, 7.8, and 2.9 Hz, H-9) and 5.19 (1H, double-heptet, J = 8.3 and 1.5 Hz, H-10) of **1** were replaced by signals at δ 3.05 (2H, s) and 2.35 (2H, d, J = 7.3 Hz). In addition, two of the vinyl methyls in the region of δ 1.71−1.61 in the ^1H NMR spectrum of **1** were shifted upfield to δ 0.90 (6H, d, J = 6.4 Hz). A combination of ^1H COSY and HSQC experiments revealed that all of the structural variation occurred in the terminal isoprene unit in that the C-10 double bond of **1** was hydrogenated and the C-9 carbinol was oxidized to a ketone. This interpretation was supported by an observation of HMBC correlations between the signals of the C-9 carbonyl carbon and neighboring protons. Thus, the structure of euplexide D (**4**) was determined as a farnesylhydroquinone galactoside possessing a ketone at C-9.

The molecular formula of euplexide E (**5**) was deduced as $C_{35}H_{52}O_{11}$ by a combined HRFABMS and ^{13}C NMR analysis. The NMR data for this compound were highly compatible with those derived from **1**−**4**. Careful examination of the ^{13}C NMR data revealed that one of the trisubstituted double bonds of other euplexides was replaced by a disubstituted one in **5**. In addition, the signal of a new methoxy carbon appeared at δ 50.2. On the basis of the results of ^1H COSY and HSQC experiments, the corresponding proton signals were found at δ 5.55 (1H, dt, J = 16.1 and 6.8 Hz), 5.41 (1H, d, J = 16.1 Hz), and 3.18 (3H, s), respectively. Also observed in the ^1H NMR spectrum was the upfield shift of the H-12 and H-15 protons of the terminal isoprene unit from δ 1.75−1.55 (**1**−**3**) to δ 1.28 (6H, s). Accordingly the locations of the double bond and the methoxy group were assigned to C-9 and C-11, respectively. This interpretation was further supported by HMBC experiments in that several two- and three-bond correlations were observed between the signals of the H-9 and H-10 olefinic protons and neighboring carbons. In addition, the attachment of the methoxy group at C-11 of the farnesyl chain was secured by a long-range correlation between the methoxy proton and the C-11 carbon. Thus, the structure of euplexide E (**5**) was determined as a derivative of euplexide A (**1**) possessing an allylic methoxy functionality.

Polyprenylated quinones (hydroquinones) and related mixed biogenetic metabolites have been frequently isolated from various marine organisms such as brown algae and sponges.[1] Among metabolites of this structural class, however, compounds containing an additional sugar moiety have been very rarely found. To the best of our knowledge, euplexides are precedented only by moritoside from the Japanese gorgonian *Euplexaura* sp.[6] As a related series of compounds, pseudopterosins and seco-pseudopterosins, the potent antiinflammatory agents from the Caribbean gorgonian *Pseudopterogorgia elisa-*

bethae, contained both an orthohydroquinone and a glycoside moiety.[12,13] However, these compounds are structurally distinct from euplexides in that the hydroquinones of pseudopterosins are derived from a geranylgeranyl chain while those of euplexides have a different biosynthetic origin.

Polyprenylated quinones and hydroquinones are widely recognized to exhibit potent and diverse bioactivities.[1] As an example, moritoside inhibited cell division in fertilized starfish eggs at the concentration of 1 μg/mL in seawater.[6] In our measurement, compounds **1**−**5** exhibited moderate cytotoxicity against the human leukemia cell-line K462 with IC_{50} values of 2.6, 3.1, 5.2, 8.1, and 9.4 μg/mL, respectively. At the concentrations of 10 μg/300 μL, **1**−**3** and **5** also displayed antioxidizing activity of 3.42, 3.56, 3.45, and 3.13 times, respectively, higher than that of superoxide dismutase (SOD). In addition, **1** and **2** exhibited 52 and 71%, respectively, inhibition of PLA_2 at the concentration of 50 μg/mL.

Experimental Section

Collection, Extraction, and Isolation. *Euplexaura anastomosans* (sample number 91K-1) was collected by hand using scuba at 15−35 m depth in November 1991 and October 1995 off the shore of Keomun Island, Korea.[14] The collection was briefly surface air-dried in the shade and kept at −25 °C until chemically investigated. The defrosted specimens were examined for chemical homogeniety by TLC analysis of each individual colony using 100% Et_2O and 50% Et_2O in *n*-hexane as two different developing solvents.[5] The chemically homogeneous specimens were combined (16.0 kg for 63 colonies), freeze-dried, macerated, and repeatedly extracted with CH_2Cl_2 (15 L × 3) and MeOH (15 L × 2). The combined crude extracts (68.5 g) were subjected to silica vacuum flash chromatography by using sequential mixtures of *n*-hexane and EtOAc as eluents. Fractions eluted with moderately polar solvents (40−45% EtOAc in hexane) were combined and separated by semipreparative silica HPLC (YMC column, 1 cm × 25 cm, 40% EtOAc in hexane) to yield compounds **1**−**5** in the order of **4**, **3**, **2**, **1**, and **5.** Final purification was established by semipreparative C_{18} reversed-phase HPLC (YMC ODS column, 1 cm × 25 cm, 15% aqueous MeOH for **3** and **4**; 20% aqueous MeOH for **1**, **2**, and **5**) to afford 51.4, 39.2, 11.0, 5.8, and 9.8 mg of **1**−**5**, respectively.

Euplexide A (1): a white solid; mp 92−93 °C; $[\alpha]^{25}_D$ −2.5° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3400, 2925, 1740, 1440, 1380, 1200, 1075 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 285 (3.49), 210 (4.15) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively. HMBC correlations: H-1/C-2, C-3, C-4′, C-5′, C-6′; H-2/C-4, C-13; H-4/C-2, C-3, C-5; H-5/C-3, C-4; H-8/C-6, C-7; H-9/C-7; H-10/C-15; Me-12/C-10, C-11, C-15; Me-13/C-2, C-3, C-4; Me-14/C-6, C-7, C-8; Me-15/C-10, C-11, C-12; H-3′/C-1′, C-4′, C-5′; H-6′/C-1, C-1′, C-2′; Me-7′/C-1′, C-2′, C-3′; H-1″/C-4′; H-2″/C-1″, C-3″; H-4″/C-2″, C-3″; H-5″/C-1″, C-4″, C-6″; H-6″/C-4″, C-5″. HRFABMS [M + Na]$^+$: *m/z* 655.3099 (calcd for $C_{34}H_{48}O_{11}$Na, 655.3094).

Euplexide B (2): a white solid; mp 170−180 °C (decomposed); $[\alpha]^{25}_D$ −7.9° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3400, 2925, 1750, 1370, 1240, 1080 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 287 (3.59), 212 (4.23) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively. HMBC correlations: H-1/C-2, C-3, C-4′, C-5′, C-6′; H-2/C-4, C-13; H-4/C-2, C-3, C-5; H-5/C-3, C-4; H-8/C-6, C-7; H-9/C-7, carbonyl(9-OAc); H-10/C-15; Me-12/C-10, C-11, C-15; Me-13/C-2, C-3, C-4; Me-14/C-6, C-7, C-8; Me-15/C-10, C-11, C-12; H-3′/C-1′, C-4′, C-5′; H-6′/C-1, C-1′, C-2′, C-4′; Me-7′/C-1′, C-2′, C-3′; H-1″/C-4′; H-2″/C-1″, C-3″; H-4″/C-2″, C-3″; H-5″/C-1″, C-4″, C-6″; H-6″/C-4″, C-5″; Me(9-OAc)/carbonyl. HRFABMS [M + Na]$^+$: *m/z* 697.3226 (calcd for $C_{36}H_{50}O_{12}$Na, 697.3200).

Euplexide C (3): a colorless gum; $[\alpha]^{25}_D$ −11.9° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3400, 2925, 1750, 1500, 1370, 1235, 1080 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 288 (3.22), 210 (4.27) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively. HMBC correlations: H-1/C-2, C-3, C-4′, C-5′, C-6′; H-2/C-4, C-13; H-4/C-2, C-3, C-5, C-6; H-6/C-8, C-14; H-8/ C-6, C-7, C-9, C-14; H-9/C-10; Me-12/C-10, C-11, C-15; Me-13/C-2, C-3, C-4; Me-14/C-6, C-7, C-8; Me-15/C-10, C-11, C-12; H-3′/C-1′, C-5′, C-7′; H-6′/C-1, C-2′, C-4′, C-5′; Me-7′/C-1′, C-2′, C-3′; H-1″/C-4′; H-2″/C-1″, C-3″; H-3″/C-2″; H-4″/C-2″, C-3″; H-5″/C-4″; H-6″/C-4″, C-5″. HRFABMS [M + Na]$^+$: *m/z* 639.3142 (calcd for $C_{34}H_{48}O_{10}$Na, 639.3146).

Euplexide D (4): a colorless gum; $[\alpha]^{25}_D$ −13.1° (*c* 0.3, $CHCl_3$); IR (KBr) ν_{max} 3400, 2960, 1750, 1710, 1430, 1370, 1235, 1080 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 287 (3.43), 212 (4.15) nm; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively. HMBC correlations: H-1/C-2, C-3, C-4′, C-5′, C-6′; H-2/C-4, C-13; H-4/C-2, C-3, C-6, C-13; H-5/C-3, C-6; H-6/C-8, C-14; H-8/C-6, C-9, C-14; H-10/C-9, C-11, C-12(−15); H-11/C-12(−15); Me-12(−15)/C-10, C-11; Me-13/C-2, C-3, C-4; Me-14/C-6, C-7, C-8; H-3′/C-1′, C-4′, C-5′, C-7′; H-6′/C-1, C-1′, C-2′, C-4′; Me-7′/C-1′, C-3′; H-1″/C-4′; H-2″/C-1″, C-3″; H-3″/C-2″, C-4″; H-4″/C-2″, C-3″; H-5″/C-1″, C-4″, C-6″; H-6″/C-4″, C-5″. HRFABMS [M + Na]$^+$: *m/z* 655.3118 (calcd for $C_{34}H_{48}O_{11}$Na, 655.3094).

Euplexide E (5): a colorless gum; $[\alpha]^{25}_D$ −12.6° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3400, 2975, 2930, 1750, 1370, 1235, 1075 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 287 (3.48), 211 (4.24) nm; ^1H and ^{13}C NMR Data, see Tables 1 and 2, respectively. HMBC correlations: H-1/C-2, C-3, C-4′, C-5′, C-6′; H-2/C-1, C-3, C-4, C-13; H-4/C-3, C-5, C-13; H-5/C-3, C-4, C-6, C-7; H-6/C-8, C-14; H-8/C-6, C-7, C-9, C-10, C-14; H-9/C-7, C-8, C-10, C-11; H-10/C-8, C-9, C-11, C-12, C-15; Me-12(−15)/C-10, C-11; Me-13/C-2, C-3, C-4; Me-14/C-6, C-7, C-8; H-3′/C-1′, C-4′, C-5′, C-7′; H-6′/C-1, C-1′, C-2′, C-4′, C-5′; Me-7′/C-1′, C-2′, C-3′; H-1″/C-4′; H-2″/C-3″; H-3″/C-2″, C-4″; H-4″/C-2″, C-3″; H-5″/C-1″, C-4″, C-6″; H-6″/C-4″, C-5″; OMe/C-11. HRFABMS [M + Na]$^+$: *m/z* 669.3253 (calcd for $C_{35}H_{50}O_{11}$Na, 669.3251).

(S)*-MTPA Ester of 1**. To a solution of **1** (3.1 mg) in dry pyridine (100 μL) was added (−)-MTPA chloride (20 μL). The mixture was allowed to stand under N_2 at room temperature for 2 h. After confirmation of the consumption of starting material by TLC, H_2O (50 μL), CH_2Cl_2 (100 μL), and MeOH (1 mL) were sequentially added to the mixture. The solvents were removed under vacuum and the residue was separated by semipreparative silica HPLC (20% EtOAc in hexane) to yield pure (*S*)-MTPA ester **1S* (1.8 mg): ^1H NMR ($CDCl_3$) δ 7.68−7.15 (15H, m, ArH), 6.79 (1H, s, H-6′), 6.72 (1H, s, H-3′), 5.82 (1H, ddd, J = 9.3, 8.3, 5.9 Hz, H-9), 5.73 (1H, dd, J = 10.7, 7.8 Hz, H-2″), 5.47 (1H, br d, J = 3.4 Hz, H-4″), 5.19 (1H, d, J = 7.8 Hz, H-1″), 5.18 (1H, m, H-6), 5.12 (1H, br t, J = 7.3 Hz, H-2), 5.11 (1H, dd, J = 10.7, 3.4 Hz, H-3″), 5.02 (1H, br d, J = 9.3 Hz, H-10), 4.19 (1H, dd, J = 11.2, 7.3 Hz, H-6″), 4.15 (1H, dd, J = 11.2, 5.9 Hz, H-6″), 4.07 (1H, br dd, J = 7.3, 5.9 Hz, H-5″), 3.67 (3H, s, OMe), 3.52 (3H, s, OMe), 3.41 (3H, s, OMe), 3.32 (1H, dd, J = 16.6, 7.3 Hz, H-1), 3.09 (1H, dd, J = 16.6, 7.3 Hz, H-1), 2.41 (1H, dd, J = 14.2, 8.3 Hz, H-8), 2.21 (1H, dd, J = 14.2, 5.9 Hz, H-8), 2.20 (3H, s, OAc), 2.06 (3H, br s, H-7′), 2.04 (2H, m, H-5), 2.03 (3H, s, OAc), 1.96 (2H, t, J = 8.3 Hz, H-4), 1.94 (3H, s, OAc) 1.747 (3H, d, J = 1.0 Hz, H-12), 1.69 (3H, br s, H-15), 1.62 (3H, br s, H-14), 1.59 (3H, br s, H-13); HRFABMS [M + Na]$^+$: *m/z* 1303.4291 (calcd for $C_{64}H_{69}F_9O_{17}$Na, 1303.4289).

(R)*-MTPA Ester of 1.** This was prepared as described for **1S*. From 3.7 mg of **1** was obtained 1.7 mg of (*R*)-MTPA ester **1***R*: ^1H NMR ($CDCl_3$) δ 7.67−7.15 (15H, m, ArH), 6.77 (1H, s, H-3′), 6.49 (1H, s, H-6′), 5.84 (1H, ddd, J = 9.3, 7.8, 5.9 Hz,

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(13) Look, S. A.; Fenical, W. *Tetrahedron* **1987**, *43*, 3363−3370.

(14) Specimens of *E. anastomosans* under code name 91K-1 are on deposit in the coelenterate collection, Natural History Museum, Ewha Womans University, Seoul, Korea, under the curatorship of Professor Jun-Im Song.

H-9), 5.70 (1H, dd, J = 10.7, 7.8 Hz, H-2″), 5.47 (1H, br d, J = 3.4 Hz, H-4″), 5.17 (1H, dd, J = 10.7, 3.4 Hz, H-3″), 5.17 (1H, t, J = 7.3 Hz, H-2), 5.16 (1H, br d, J = 9.3 Hz, H-10), 5.11 (1H, br t, J = 7.3 Hz, H-6), 5.05 (1H, d, J = 7.8 Hz, H-1″), 4.14 (2H, d, J = 6.8 Hz, H-6″), 4.00 (1H, br t, J = 6.8 Hz, H-5″), 3.67 (3H, s, OMe), 3.52 (3H, s, OMe), 3.48 (3H, s, OMe), 3.29 (1H, dd, J = 16.6, 7.3 Hz, H-1), 3.23 (1H, dd, J = 16.6, 7.3 Hz, H-1), 2.36 (1H, dd, J = 14.2, 7.8 Hz, H-8), 2.19 (3H, br s, H-7′), 2.16 (1H, dd, J = 14.2, 5.9 Hz, H-8), 2.02 (3H, s, OAc), 2.01 (2H, m, H-4), 1.99 (3H, s, OAc), 1.99 (2H, m, H-5), 1.98 (3H, s, OAc), 1.754 (3H, d, J = 1.0 Hz, H-12), 1.73 (3H, br s, H-15), 1.64 (3H, br s, H-13), 1.56 (3H, br s, H-14); HRFABMS [M + Na]$^+$: m/z 1303.4303 (calcd for $C_{64}H_{69}F_9O_{17}Na$, 1303.4289). Δ(δ**1**S−δ**1**R): H-6, +37 Hz; H-8, +27 and +22 Hz; H-10, −71 Hz; H-12, −3 Hz; H-14, +31 Hz; H-15, −20 Hz; H-1″, +70 Hz; H-3″, −30 Hz; H-4″, −4 Hz; H-5″, + 36 Hz; H-6″, +19 and +3 Hz.

Acetylation and Deacetylation of 1. To a stirred solution of euplexide A (7.1 mg) in dry pyridine (0.5 mL) was added acetic anhydride (0.2 mL). After the mixture was stirred at room temperature for 1 h, the solvent and excess reactant were removed under vacuum, and the residue was redissolved in 2 M methanolic ammonia (3 mL). After the mixture was stirred for 5 h at room temperature, the solvent was removed by blowing with N_2 to yield pure 9-acetyl-3″, 4″, 6″-trideacetylated euplexide A (**7**): ^{1}H NMR ($CDCl_3$) δ 6.81 (1H, br s, H-3′), 6.55 (1H, s, H-6′), 5.60 (1H, ddd, J = 9.3, 7.8, 7.8 Hz, H-9), 5.20 (1H, dd, J = 7.3, 6.8 Hz, H-2), 5.15 (1H, dd, J = 6.8, 6.8 Hz, H-6), 5.13 (1H, br d, J = 9.3 Hz, H-10), 4.64 (1H, d, J = 7.8 Hz, H-1″), 4.07 (1H, br s, H-4″), 3.93−3.83 (3H, m, H-2″, H-6″), 3.67 (1H, br d, J = 9.3 Hz, H-3″), 3.54 (1H, br dd, J = 5.4, 4.9 Hz, H-5″), 3.33 (1H, dd, J = 16.1, 7.3 Hz, H-1), 3.24 (1H, dd, J = 16.1, 6.8 Hz, H-1), 2.32 (1H, dd, J = 13.7, 7.8 Hz, H-8), 2.14 (3H, br s, H-7′), 2.11−2.01 (5H, m, H-4, H-5, H-8), 1.99 (3H, s, OAc), 1.71 (3H, s, H-12), 1.67 (3H, s, H-15), 1.61 (3H, br s, H-14), 1.59 (3H, s, H-13); HRFABMS [M + Na]$^+$: m/z 571.2882 (calcd for $C_{30}H_{44}O_9Na$, 571.2883).

Acid Hydrolysis of 7. A portion of **7** (1 mg) was dissolved in 1 N HCl (0.2 mL), and the solution was stirred at 50 °C for 3 h. After removal of the solvent by blowing with N_2, Trisil-Z (0.2 mL) was added to the residue, which was then heated at 60 °C for 1 min. After the mixture was dried with a stream of N_2, the residue was redissolved in CH_2Cl_2 (0.2 mL) and analyzed by GC using a Chirasil-Val column (0.32 mm × 25 m). Temperatures of injector and detector were 200 °C. A temperature gradient system was used for the oven in that the initial temperature was maintained at 60 °C for 3 min and then raised to 200 °C in the rate of 4 °C/min. A peak of a sugar product was detected at 20.6667 min. Retention times for authentic samples after treated simultaneously with Trisil-Z were 20.7167 and 21.0167 min for D- and L-galactose, respectively. Co-injection of the hydrolysis product with authentic samples gave a single peak of D-galactose at 20.6724 min.

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Supporting Information Available: Copies of the ^{1}H NMR spectra of **1**−**5**, **1**S, and **1**R, ^{13}C NMR spectra of **1**−**5**, and ^{1}H COSY, HSQC, and HMBC spectra of **1** (15 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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