

NEW FARNESYLHYDROQUINONE GLYCOSIDES FROM THE GORGONIAN *EUPLEXAURA ANASTOMOSANS*.

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Abstract: Euplexides F (3) and G (4), novel farnesylhydroquinone glycosides, have been isolated as minor constituents of the gorgonian *Euplexaura anastomosans*. The structures of these compounds have been determined by combined spectroscopic methods. These compounds exhibited moderate cytotoxicity and inhibitory activity against PLA₂.

Key Words: Euplexides F and G, farnesylhydroquinone glycosides, gorgonian, *Euplexaura anastomosans*.

INTRODUCTION

Coelenterates of the order alcyonacea (soft corals) and gorgonacea (gorgonians) are widely recognized as very prolific sources of bioactive secondary metabolites.^{1,2} During the course of chemical investigation of benthic invertebrates of Korean waters, we recently reported the structures of euplexides A-E, novel farnesylhydroquinone glycosides of the moritoside class from the gorgonian *Euplexaura anastomosans*, collected from Keomun Island.^{3,4} These compounds exhibited cytotoxicity and antioxidizing activity as well as inhibitory activity against PLA₂.

In our continuing search for bioactive substances from marine organisms, we re-encountered *E. anastomosans* from the nearby Bacdo. The large-scale extraction of organic materials followed by bioactivity-guided separation using combined chromatographic techniques yielded euplexides A-E, together with two additional metabolites of the same structural class. Herein we report the structures and bioactivities of these minor farnesylhydroquinone glycosides.

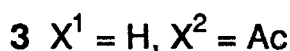
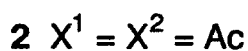
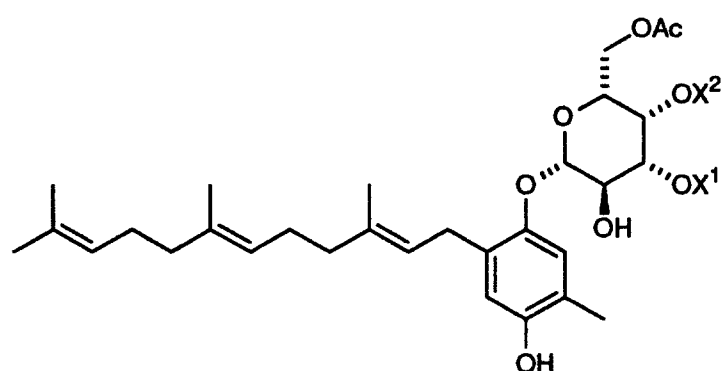
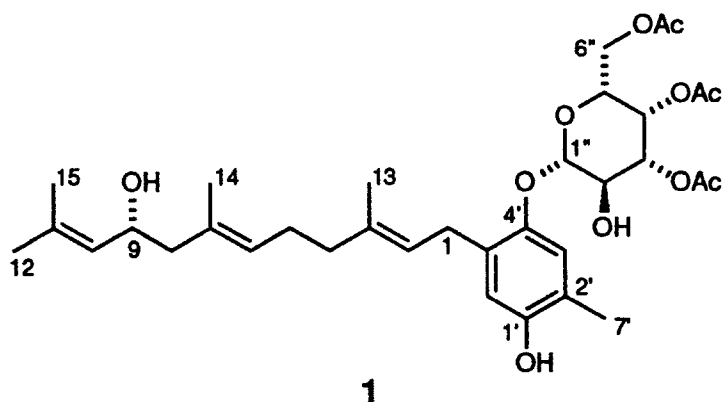
RESULTS AND DISCUSSION

Separation of the moderately polar fractions from silica vacuum flash chromatography of the crude extract using HPLC has yielded euplexides A-E. As observed previously, euplexides A (1) and B were the major metabolites; concentrations of these were an order of magnitude higher than those of other metabolites.⁴

Euplexide F (3) was isolated as a colorless gum which analyzed for $C_{32}H_{46}O_9$ by combined HRFABMS and ^{13}C NMR spectrometry. The spectral data of this compound were highly compatible with those obtained for other euplexides. The only significant difference in the NMR data was the disappearance of signals corresponding to an acetyl group; $\delta_H \sim 2.1$ (3H, s), $\delta_C \sim 170$ (C) and 21 (CH_3). Furthermore, compound 3 was found from combined 2-D NMR analysis to be identical to euplexide C (2) with respect to the farnesyl chain and 1,4-hydroquinone moiety. The connection between these partial structures at C-1/C-5' was proved by HMBC correlations of H-1 and H-6' with neighboring carbons. Additional data supporting this interpretation was provided by a NOESY experiment in which strong cross peaks were observed for H-1/H-6' and H-2/H-6'.

The structure of the sugar portion was also determined by 2-D NMR experiments. Based upon the 1H NMR and 1H COSY data, the sugar protons were sequentially assigned at δ 4.69 (anomeric), 3.90, 3.86, 5.38, 3.97, and 4.20 (2H), respectively. The downfield shifts of H-4" and H-6" at δ 5.38 and 4.20, respectively, as well as the HMBC correlations of these protons with the carbonyl carbons revealed the attachment of the acetyl groups at these positions. The

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HMBC data also showed a long-range correlation of the anomeric C-1'' with H-5'' indicating the pyranose nature of the sugar. On the basis of vicinal proton-proton coupling constants, the orientations of H-1''~H-4'' were assigned as axial, axial, axial, and equatorial, respectively; $J_{1'',2''} = 7.3$ Hz, $J_{2'',3''} = 9.8$ Hz, $J_{3'',4''} = 3.4$ Hz. Due to the small vicinal coupling constant ($J_{4'',5''} =$ broad singlet), however, the orientation of H-5'' was not determined by the ^1H NMR data. The mutual NOESY correlations among H-1'', H-3'', and H-5'' allowed to assign an axial orientation of this proton. Thus, the sugar moiety of **3** was defined as a β -4,6-diacetyl-galactopyranose. Although the absolute configuration of the sugar moiety was not directly determined by chemical degradation and GC analysis, the similar specific rotation ($[\alpha]^{25}_{\text{D}} -15.1^\circ$) suggested the galactose of **3** to have D configuration.⁴ Finally the glycosidic linkage between the anomeric carbon of sugar and C-4' of hydroquinone was defined on the basis of the HMBC correlation

for H-1"/C-4' and a NOESY cross peak for H-3'/H-1". Thus, the structure of euplexide F (3) was determined as the 3"-deacetylated derivative of euplexide C (2).

The molecular formula of euplexide G (4) was deduced as C₃₂H₄₆O₉, identical to that of euplexide F by HREIMS and ¹³C NMR spectrometry. The spectral data of this compound were also very similar to those of 3. The ¹H and ¹³C NMR data showed that 4 had the same farnesyhydroquinone portion as 3. However, careful examination of the NMR data revealed that significant differences occurred within the signals of protons and carbons of the sugar moiety. The ¹H COSY traced a proton spin system consisted of signals at δ 4.73 (anomeric), 4.09, 4.92, 4.07, 3.83, and 4.36 (and 4.32, geminal). The downfield shifts of H-3" and H-6" as well as the HMBC correlations of these with the carbonyl carbons assigned the attachment of acetyl groups at C-3" and C-6". The β-galactopyranose nature of the sugar was defined by a combination of ¹H-¹H coupling constant analysis, HMBC (H-5"/C-1") and NOESY (H-1"/H-3", H-1"/H-5", H-3"/H-5") data. Similarly, the glycosidic linkage between the sugar and hydroquinone was also determined by combined 2-D NMR data. Thus, the structure of euplexide G (4) was defined as the 4"-deacetylated derivative of euplexide C (2).

Moritoxide, the first metabolite in this series isolated from the Japanese gorgonian *Euplexaura* sp., was reported to inhibit cell division in fertilized starfish eggs.³ Euplexides A-E exhibited cytotoxicity against the human leukemia cell-line K562 with LC₅₀ values in the range of 2-10 μg/mL.⁴ In addition, euplexides A and B displayed antioxidizing activity of SOD type and significant inhibition of PLA₂. In our measurement with the newly isolated metabolites, 3 and 4 were also cytotoxic against K562 cell-line with LC₅₀ values 8.7 and 11.3 μg/mL, respectively. compounds 3 and 4 also exhibited 47 and 58%, respectively, inhibition of PLA₂ at the concentration of 50 μg/mL.

EXPERIMENTAL

Instruments. The optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in methanol using a Milton-Roy

spectrophotometer. NMR spectra were recorded in CDCl_3 solutions on a Varian Unity-500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si . Mass spectra were obtained by using a Jeol JMS-HX 110 mass spectrometer and provided by Korea Basic Science Institute, Taejeon, Korea.

Animal material. Samples of the gorgonian *Euplexaura anastomosans* (sample number 98B-1) were collected by hand at 10-20 m depth in April, 1998 off the coast of Bacdo, Korea. Morphological characters of the specimens were identical to those described previously for another *E. anastomosans* collected from the nearby Keomun Island.³

Extraction and isolation. The specimens were immediately frozen on dry ice and stored at -25°C until investigated chemically. The defrosted animals were briefly dried under shade (19.1 kg) and repeatedly extracted with CH_2Cl_2 (16 L x 3) and MeOH (16 L x 1). The combined crude extracts (151.8 g) were subjected to silica vacuum flash chromatography using stepped gradient mixtures of *n*-hexane and EtOAc as eluents. Fractions eluted with moderately polar solvents (40-50% EtOAc/hexane) were combined and separated by semipreparative C_{18} reversed-phase HPLC (YMC ODS-A column, 30% aqueous MeCN) to yield in the order of elution euplexides C (2), D, B, E, and A (1); 328.4, 203.1, 42.6, 87.0, and 20.4 mg for euplexides A-E, respectively.

The fractions eluted with polar solvents (90-100% EtOAc/hexane) were combined and separated by reversed-phase HPLC (45% aqueous MeCN) to afford euplexides F (3) and G (4). Final purification was accomplished by reversed-phase HPLC (YMC ODS-AQ column, 20% aqueous MeOH) to afford 10.3 and 6.5 mg of 3 and 4, respectively.

Euplexide F (3): colorless gum; $[\alpha]_D^{25} -15.1^\circ$ (*c* 0.07, MeOH); IR (KBr) ν_{max} 3400, 2930, 1745, 1500, 1440, 1370, 1240 cm^{-1} ; UV (MeOH) λ_{max} (log ϵ) 288 (3.05), 210 (4.32) nm; ^1H NMR (CDCl_3) δ 6.86 (1H, s, H-3'), 6.57 (1H, s, H-6'), 5.38 (1H, br d, $J = 3.4$ Hz, H-4''), 5.25 (1H, br dd, $J = 7.8, 5.4$ Hz, H-2), 5.11 (1H, br t, $J = 7.0$ Hz, H-6), 5.09 (1H, br t, $J = 7.0$ Hz, H-10), 4.69 (1H, d, $J = 7.3$ Hz, H-1''), 4.20 (2H, d, $J = 6.4$ Hz, H-6''), 3.97 (1H, br t, $J = 6.4$ Hz, H-5''), 3.90 (1H, dd, $J = 9.8, 7.3$ Hz, H-2''), 3.86 (1H, dd, $J = 9.8, 3.4$ Hz, H-3''), 3.44 (1H, dd, $J = 16.1, 7.8$ Hz, H-1), 3.19 (1H, dd, $J = 16.1, 5.4$ Hz, H-1),

2.20 (3H, s, H-7'), 2.18 (3H, 4''-OAc), 2.10 (2H, m, H-5), 2.07 (3H, s, 6''-OAc), 2.04 (4H, m, H-4, H-9), 1.97 (2H, dd, $J = 8.3, 6.8$ Hz, H-8), 1.72 (3H, br s, H-13), 1.67 (3H, br s, H-12), 1.60 (3H, s, H-15), 1.59 (3H, s, H-14); ^{13}C NMR (CDCl_3) δ 171.0 (C, 4''-OAc), 170.5 (C, 6''-OAc), 149.4 (C, C-1'), 149.0 (C, C-4'), 136.8 (C, C-3), 135.2 (C, C-7), 131.4 (C, C-11), 130.2 (C, C-5'), 124.3 (CH, C-10), 123.9 (CH, C-6), 122.9 (CH, C-2), 121.9 (C, C-2'), 119.0 (CH, C-3'), 116.2 (CH, C-6'), 102.9 (CH, C-1''), 72.0 (CH, C-3''), 71.7 (CH, C-2''), 71.3 (CH, C-5''), 68.9 (CH, C-4''), 62.1 (CH_2 , C-6''), 39.7 (CH_2 , C-4), 39.6 (CH_2 , C-8), 28.7 (CH_2 , C-1), 26.7 (CH_2 , C-9), 26.5 (CH_2 , C-5), 25.7 (CH_3 , C-12), 20.8 (CH_3 , 4''-OAc), 20.7 (CH_3 , 6''-OAc), 17.7 (CH_3 , C-15), 16.2 (CH_3 , C-13), 16.0 (CH_3 , C-14), 15.8 (CH_3 , C-7'); HRFABMS m/z obsd 597.3060 $[\text{M}+\text{Na}]^+$; calcd for $\text{C}_{32}\text{H}_{46}\text{O}_9\text{Na}$, 597.3040 ($\Delta+2.0$ mmu).

Euplexide G (4): colorless gum; $[\alpha]_D^{25}$ -21.6° (c 0.12, MeOH); IR (KBr) ν_{max} 3400, 2950, 1745, 1400, 1370, 1240 cm^{-1} ; UV (MeOH) λ_{max} ($\log \epsilon$) 287 (3.55), 210 (4.52) nm; ^1H NMR (CDCl_3) δ 6.88 (1H, s, H-3'), 6.57 (1H, s, H-6'), 5.24 (1H, br dd, $J = 7.6, 6.4$ Hz, H-2), 5.11 (1H, tq, $J = 6.6, 1.0$ Hz, H-6), 5.09 (1H, tq, $J = 6.8, 1.5$ Hz, H-10), 4.92 (1H, dd, $J = 10.3, 3.4$ Hz, H-3''), 4.73 (1H, d, $J = 7.8$ Hz, H-1''), 4.36 (1H, dd, $J = 11.7, 5.9$ Hz, H-6''), 4.32 (1H, dd, $J = 11.7, 6.8$ Hz, H-6''), 4.09 (1H, dd, $J = 10.3, 7.8$ Hz, H-2''), 4.07 (1H, dd, $J = 3.4, 1.0$ Hz, H-4''), 3.83 (1H, br dd, $J = 6.8, 5.9$ Hz, H-5''), 3.41 (1H, dd, $J = 16.1, 7.6$ Hz, H-1), 3.21 (1H, dd, $J = 16.1, 6.4$ Hz, H-1), 2.20 (3H, s, H-7'), 2.18 (3H, s, 4''-OAc), 2.10 (2H, m, H-5), 2.09 (3H, s, 6''-OAc), 2.05 (4H, m, H-4, H-9), 1.97 (2H, dd, $J = 8.3, 6.8$ Hz, H-8), 1.69 (3H, br s, H-13), 1.67 (3H, br s, H-12), 1.60 (3H, s, H-15), 1.59 (3H, s, H-14); ^{13}C NMR (CDCl_3) δ 170.8 (C, 6''-OAc), 170.5 (C, 4''-OAc), 149.5 (C, C-1'), 149.0 (C, C-4'), 136.8 (C, C-3), 135.2 (C, C-7), 131.4 (C, C-11), 130.4 (C, C-5'), 124.3 (CH, C-10), 124.0 (CH, C-6), 122.6 (CH, C-2), 121.9 (C, C-2'), 119.4 (CH, C-3'), 116.1 (CH, C-6'), 103.4 (CH, C-1''), 74.6 (CH, C-3''), 72.3 (CH, C-5''), 69.2 (CH, C-2''), 67.0 (CH, C-4''), 62.4 (CH_2 , C-6''), 39.7 (CH_2 , C-4), 39.6 (CH_2 , C-8), 28.4 (CH_2 , C-1), 26.7 (CH_2 , C-9), 26.5 (CH_2 , C-5), 25.7 (CH_3 , C-12), 21.0 (CH_3 , 4''-OAc), 20.8 (CH_3 , 6''-OAc), 17.7 (CH_3 , C-15), 16.2 (CH_3 , C-13), 16.0 (CH_3 , C-14), 15.8 (CH_3 , C-7'); HREIMS m/z obsd 574.3145 $[\text{M}]^+$; calcd for $\text{C}_{32}\text{H}_{46}\text{O}_9$, 574.3142 ($\Delta+0.3$ mmu).

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