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Octa- and Nonaprenylhydroquinone Sulfates, Inhibitors of α1,3-Fucosyltransferase VII, from an Australian Marine Sponge *Sarcotragus* sp.[1]

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Abstract: α1,3-Fucosyltransferase (Fuc TVII) is a key enzyme in the biosynthesis of selectin ligands. We have isolated two inhibitors of Fuc TVII from a marine sponge *Sarcotragus* sp. They were characterized as octa- and nonaprenylhydroquinone sulfates on the basis of spectral data. These compounds inhibited Fuc-TVII with IC_{50} values of 3.9 and 2.4 μg/mL, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords : Enzyme inhibitor; Marine metabolites; Natural products; Terpenes and terpenoids

Introduction

α1,3-Fucosyltransferases (Fuc Ts) which catalyze the transfer of L-fucopyranoside residues from guanosine diphosphate fucose (GDP-fucose) to glycoconjugate acceptors are known to be involved in the biosynthesis of the sialyl Lewis X (SLe^x : Neu5Acα(2→3)-Galβ(1→4)[Fucα(1→3)GlcNAc]) present on the extracellular surfaces of leukocytes.[2] SLe^x serves as a common ligand for E-, L-, and P-selectins, endothelial cell-adhesion glycoproteins.[3] The E-selectin-SLe^x interaction encourages leukocytes to move from the bloodstream into sites of injury or infection, thus causing inflammation.[4] Therefore, inhibitors of α1,3-fucosyltransferase, especially Fuc TVII are potential drugs for the treatment of inflammatory diseases.

In the course of our search for enzyme inhibitors which are potential drugs, we tested more than 1,000 extracts of marine sponges for inhibition of Fuc TVII and Fuc TVI using soluble recombinant enzymes[5,6] which resulted in the discovery of 63 active species. Notably,

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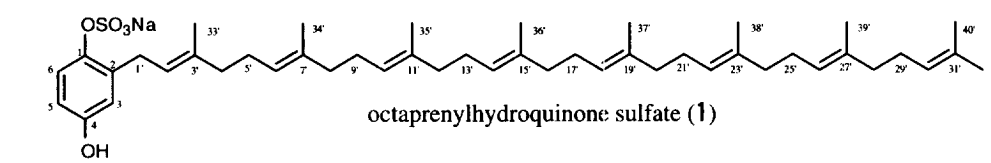
the lipophilic extract of an Australian *Sarcotragus* sp. showed promising activity. Bioassay-guided isolation afforded two active compounds which were prenylhydroquinone sulfates. We describe the isolation, structure elucidation, and activity of these compounds.

Results and Discussion

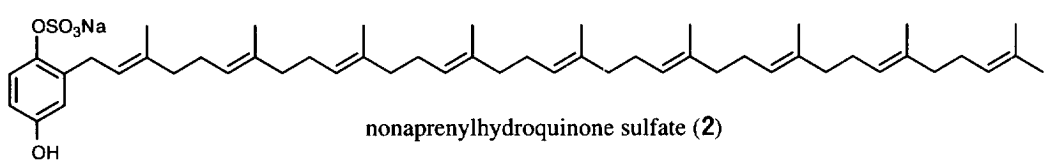
The Et_2O-soluble portion of the MeOH and $CHCl_3$/MeOH (1:1) extracts of the frozen sponge (150 g wet weight) was partitioned between hexane and 90% MeOH. The latter layer was fractionated by ODS flash chromatography followed by ODS HPLC to yield compound **1** (45.5 mg, 3.0×10^{-2} % on the basis of wet weight) and compound **2** (35.6 mg, 2.4×10^{-2} %) both as yellow amorphous powders.

Compound **1** had a molecular formula of $C_{46}H_{69}O_5SNa$ which was established by HRFABMS [$(M\text{-}Na)^-$, *m/z* 733.4874, Δ +0.9 mmu]. The UV spectrum [λ_{max} 280 nm (ε 2500)] was reminiscent of a hydroquinone chromophore, while IR absorptions at 1230 and 800 cm^{-1} indicated the presence of a sulfate group. The ^1H NMR spectrum exhibited three mutually-coupled aromatic protons attributable to a monosubstituted hydroquinone [δ 6.60 (1H, d, *J* =3.0 Hz, H-3), 6.52 (1H, dd, *J* =3.0, 9.0 Hz, H-5), 7.18 (1H, d, *J* =9.0 Hz, H-6)]; the chemical shift of 7.18 ppm was characteristic of a proton adjacent to an *O*-sulfate moiety.[7] The ^{13}C NMR spectrum revealed two oxygenated sp^2 carbon signals [144.2 (C-1), 155.5 (C-4)]; the former was sulfated, which was in good agreement with ^1H NMR data. The ^1H NMR spectrum also contained several sets of signals: an olefin coupled to a methylene which was in turn coupled to another allylic methylene; a vinylic methyl long-range coupled to an olefinic proton. These NMR features are consistent with a polyprenylhydroquinone sulfate. The sulfate ester could be placed adjacent to the prenyl chain on the basis of HMBC correlations between H-6/C-2 and H-1'/C-2. The length of the prenyl chain was inferred from the molecular formula.

Compound **2** showed spectral data almost superimposable on those of compound **1**. The negative ion HRFABMS [$(M\text{-}Na)^-$, *m/z* 801.5436, $C_{51}H_{77}O_5S$ (Δ -5.5 mmu)] indicated nine prenyl units were present in **2**. Thus, the structure is as shown.



octaprenylhydroquinone sulfate (**1**)



nonaprenylhydroquinone sulfate (**2**)

Compounds **1** and **2** inhibited Fuc-TVII with IC_{50} values of 3.9 and 2.4 μg/mL, respectively, while they showed very weak activity against Fuc-TVI as shown in Fig. 1. At present no natural products have been reported to be inhibitory against Fuc Ts. Prenylhydroquinone sulfates of marine sponge origin have shown a variety of biological activities, including ichthyotoxic, protein tyrosine kinase inhibitory and H,K-ATPase inhibitory activities.[7,8]

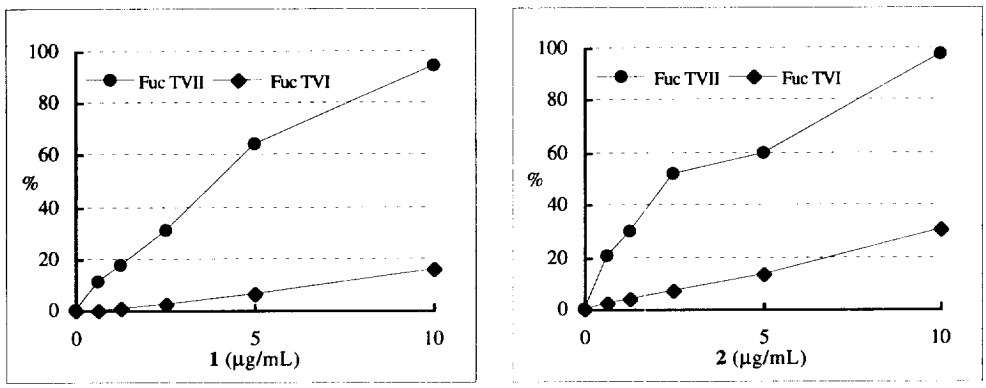


Fig.1 Inhibition of Fuc TVII and TVI by compounds **1** and **2**.

Experimental

General procedures : Ultraviolet spectra were recorded on a Hitachi 330 spectrophotometer. IR spectra were measured with a JASCO FT/IR-5300 infrared spectrometer. FAB mass spectra were measured with a JEOL SX-102 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-A600 NMR spectrometer.

Sponge sample : The sponge sample was collected on 5 September 1989 by otter trawl in Cleveland Bay, Townsville, at 15 m depth, in 15-20 minute shots. Latitude: 19° 07' S; longitude: 146° 55' E. Environment: mud/sand bottom. It was identified as *Sarcotragus* sp. (Dictyoceratida; Irciniidae) by Dr. John Hooper. The voucher was deposited at the Queensland Museum [NCI Q66C-3067-Y (QM G311135)].

Extraction and isolation : The frozen sponge (150 g) was homogenized and extracted with MeOH (1 L × 2) and $CHCl_3$/MeOH (1:1, 1 L × 2). The combined extracts were concentrated and partitioned between Et_2O and H_2O. The Et_2O-soluble portion was partitioned between *n*-hexane and 90% MeOH. The latter layer (530 mg) was fractionated by ODS flash chromatography with increasing amounts of MeOH in water. The fraction eluted with 100% MeOH was purified by ODS HPLC on Capcell Pak UG80 with 93% MeOH. The two active peaks were further purified by ODS HPLC on the same column with 100% MeOH containing 100 mM $NaClO_4$ to yield **1** (35.8 mg) and **2** (25.6 mg).

1 : yellow solid; IR ($CHCl_3$) 3040, 2920, 1230, 800 cm^{-1}, UV λ_{max}(MeOH) 280 nm (ε 2500); HRFABMS [(M-Na)$^-$, *m/z* 733.4874, $C_{46}H_{69}O_5S$ (Δ +0.9 mmu)]; ^{1}H NMR (600 MHz,

CD_3OD); δ 7.18 (1H, d,*J* =9.0 Hz, H-6), 6.60 (1H, d,*J* =3.0 Hz, H-3), 6.52 (1H, dd,*J* =3.0, 9.0 Hz, H-5), 5.32 (1H, H-2'), 5.09 (7H), 3.43 (2H, H-1'), 2.12 (2H, H-4'), 2.07 (12H), 2.05 (2H, H-4'), 1.97 (12H), 1.71 (Me-33'), 1.65 (Me-32'), 1.58 (21H); ^{13}C NMR (150 MHz, CD_3OD); 155.5 (C-4), 144.2 (C-1), 137.3 (C-3'), 137.0 (C-2), 135.9 (C-7'), 131.9 (C-31'), 125.5 (C-6'), 125.5 (C-30'), 123.9 (C-6), 123.7 (C-2'), 116.7 (C-3), 113.6 (C-5), 40.9 (C-4'), 40.9 (C-8'), 29.3 (C-1'), 27.6 (C-5'), 27.6 (C-9'), 26.0 (C-32'), 17.5 (C-40'), 16.2 (C-33'), 16.1 (C-34').

2 : yellow solid; IR ($CHCl_3$) 3040, 2920, 1230, 800 cm^{-1}, UV λ_{max}(MeOH) 278 nm (ε 2500); HRFABMS [$(M-Na)^-$, *m/z* 801.5436, $C_{51}H_{77}O_5S$ (Δ -5.5 mmu)].

Enzyme assay : Soluble recombinant Fuc TVII and Fuc TVI were prepared as described previously.[6] Standard α1,3-fucosyltransferase assay was performed in a total volume of 30 μL of 100 mM cacodylate buffer (pH 7.5), 25 mM $MnCl_2$, 0.05 mM GDP-fucose, 0.025 mM pyridylaminated α2,3-sialyl lacto-*N*-neotetraose [Neu5Acα(2→3)Galβ(1→3)GlcNAc(1→3)-Galβ(1→4)Glc], and one of the recombinant enzymes. After incubation at 37 °C for 2 h, the reaction was stopped by boiling for 5 min. After centrifugation, the supernatant was analyzed by HPLC on an ODS column (YMC-pack ODS-AQ, 6 × 150 mm, YMC, Japan) with 20 mM NH_4OAc (pH 4.0) at a flow rate of 1.0 mL/min. Eluates were monitored by fluorescence.

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