Poipuol, a New Metabolite from a Hawaiian Sponge of the Genus *Hyrtios*

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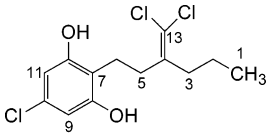
A new metabolite, poipuol (**1**), was isolated from an undescribed marine sponge *Hyrtios* sp. collected in Kauai Island, Hawaii. The structure was determined from spectroscopic data.

As part of our ongoing search for new bioactive metabolites from marine organisms, it was found that a crude extract of the Hawaiian sponge *Hyrtios* sp. (order Dictyoceratida, family Thorectidae) exhibited significant antituberculosis activity and mild cytotoxicity. Bioassay-guided fractionation led to the isolation of a known antituberculosis agent, puupehenone.[1,2] However, one of the biologically inactive chromatographic fractions obtained from the *Hyrtios* sp. extract contained a new trichlorinated metabolite, poipuol (**1**), the structure of which is described below.



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Hyrtios sp. was collected using scuba off Brenneke's Ledge, Kauai Island, Hawaii, at a depth of 18 m. The freeze-dried sponge was repeatedly extracted with 2-propanol/dichloromethane (1:1). The combined crude extract was concentrated in vacuo and partitioned between hexanes and 80% aqueous MeOH. The resulting aqueous MeOH layer was further partitioned with CH_2Cl_2. Repeated fractionation of the CH_2Cl_2-soluble materials using vacuum liquid chromatography, Sephadex LH-20, and reversed-phase HPLC provided a pure sample of compound **1** (1.2 mg).

The HREIMS spectra of poipuol (**1**) gave an $[M]^+$ ion corresponding to the molecular formula $C_{13}H_{15}Cl_3O_2$ requiring five degrees of unsaturation. The ^1H NMR [δ 6.40 (s, 2H)] and ^{13}C NMR spectra [δ 112.71 (C), δ 155.14 (2C), δ 108.43 (2CH), δ 131.9 (C)] indicated the presence of a 2,4,6-trisubstituted phenyl ring. The chemical shifts suggested that two oxygen atoms are attached to the carbons resonating at δ 155.14 of the phenyl ring (partial structure **a**). Two exchangeable protons observed at δ 1.69 and 5.11 indicated that these two oxygen atoms are both hydroxyl groups. $^1H-^1H$ COSY data established a two-methylene system (partial structure **b**) and an *n*-propyl chain (partial structure **c**). Hence the two remaining carbons resonating at δ 138.97 (C-4) and 115.57 (C-13) were assigned as a tetrasubstituted double bond (partial structure **d**), which accounted for the last degree of unsaturation. HMBC data were used to connect these partial structures as well as confirm the above structural assignments. Partial structures **a** and **b** were connected based on correlations from

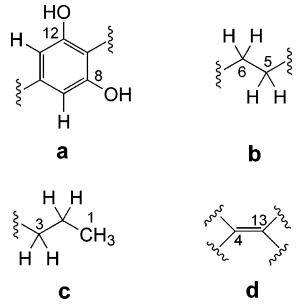

Figure 1. Partial structures of poipuol (**1**).

the methylene protons at δ 2.72 (H_2-6) in partial structure **b** to C-8 and C-12 (δ 155.14) in partial structure **a**. Three-bond correlations from C-4 (δ 138.97) to the methylene protons at δ 2.72 (H_2-6) and 1.51 (H_2-2) placed partial structure **d** between partial structures **b** and **c**. Finally, by considering the molecular formula of **1**, which required three chlorine atoms, and the chemical shift of C-13 (δ 115.57), two of the three chlorine atoms were placed on the exo-methylene carbon.[3] Thus, the last chlorine atom was assigned to C-10, completing the structure of poipuol (**1**).

Marine sponges of the genus *Hyrtios* have typically yielded secondary metabolites that are terpenoids, including mainly sesterterpenes,[4] sesquiterpene/quinones,[1,5] and tryptamine-derived alkaloids.[6] The only group of polyketide-derived metabolites isolated from *Hyrtios* sp. are the antitumor macrolides, the altohyrtins.[7] Structurally, poipuol is a small polyketide-derived compound that is unique to the genus *Hyrtios*. More interestingly, poipuol also possess the dichloro-exo-methylene moiety, which is not commonly found in natural products. However, the malyngamides[8] and the jamaicamides,[9] metabolites isolated from various collections of the marine cyanobacterium *Lyngbya majuscula*, contain a similar structural feature, the exo-chloromethylene moiety. This observation suggested the possibility of a cyanobacterial origin for poipuol. It is also worth noting a potential fungal origin of poipuol since other small polyketide-derived natural products have been recovered from the saltwater culture of *Aspergillus niger* obtained from a Caribbean collection of *H. proteus*.[10] Reexamination by light microscopy of the historical sections of the sponge specimen revealed no evidence of obvious symbiotic cyanobacteria or fungi.

Experimental Section

General Experimental Procedures. The ultraviolet spectrum was recorded on a Hewlett-Packard 8452A diode array spectrometer. The IR spectrum was recorded on a Perkin-Elmer 1600 FTIR. ^1H and ^{13}C NMR spectra were recorded in

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Table 1. NMR Spectral Data for Poipuol (**1**) in $CDCl_3$

C/H no.	δ_C[a]	δ_H (J in Hz)	HMBC[b]
1	13.93 CH_3	0.93 t (7.4)	H_2-2, H_2-3
2	20.45 CH_2	1.50 sextet	H_3-1, H_2-3
3	35.65 CH_2	2.26 t (7.7)	H_3-1, H_2-2, H_2-5
4	138.97 C		H_2-2, H_2-5, H_2-6
5	32.38 CH_2	2.41 t (8.4)	H_2-3, H_2-6
6	20.50 CH_2	2.72 t (7.9)	H_2-5
7	112.71 C		H_2-5, H_2-6
8, 12	155.14 C		H_2-6, H-9, H-12
9, 11	108.43 CH_2	6.40 s	H-9, H-11
10	131.92 C		H-9, H-11
13	115.57 C		H_2-3, H_2-5
OH		1.69 br s	
		5.11 br s	

[a] Number of attached protons deduced from the HSQC spectrum. [b] Protons showing long-range correlation with indicated carbon.

$CDCl_3$ at 500 and 125 MHz, respectively, using residue solvent signals as internal references. The HSQC experiment was optimized for $^1J_{CH} = 140$ Hz, and the HMBC experiment for 7 Hz. Mass spectral data were measured on a VG70ZAB2SE mass spectrometer. Merck aluminum-backed thin-layer chromatography sheets were used for TLC, and all solvents were distilled from glass prior to use.

The Sponge Specimen. The sponge specimen was collected using scuba off Brenneke's Ledge, Kauai Island, Hawaii, at 18 m depth, on July 27, 2003. The sponge is a massive thickly encrusting mound, and the surface is ridged with a very irregular honeycomb pattern. The ridges are pale where thick fibers packed with sand grains protrude through the unarmored ectosome. The mesohyl between the thick abundantly packed fibers is dense, uniform, and very fleshy. The color in life is dull yellow with olive tinges in preservative. The sponge is an undescribed species of *Hyrtios* (order Dictyoceratida, family Thorectidae). Voucher samples have been deposited in the Natural History Museum, London (BMNH 2004.5.27.6), and one is retained at the Department of Chemistry, University of Hawaii (072703-BREN-3).

Extraction and Isolation. Freshly collected sponge was frozen on site and transported to Oahu Island over dry ice. A freeze-dried sample of sponge (441 g) was immersed in dichloromethane and 2-propanol (1:1) and subsequently extracted repeatedly with dichloromethane and 2-propanol (1:1, 3 × 500 mL) at RT. The combined extracts were concentrated in vacuo. The crude extract was partitioned between hexanes and 80% MeOH in H_2O. The aqueous MeOH layer was subsequently extracted with dichloromethane. The dichloromethane layer (14.3 g) was subjected to silica gel vacuum liquid chromatography using stepwise gradient elution (hexanes/EtOAc/CH_2Cl_2/MeOH). The fraction eluted with CH_2Cl_2/MeOH (1:1) was further fractionated by LH-20 to provide a total of 12 fractions. Reversed-phase HPLC (Cosmosil 5C18-AR, 20 mm × 250 mm) with gradient elution (85% MeOH in H_2O to 100% MeOH) gave pure poipuol (**1**, 1.2 mg).

Poipuol (1): colorless amorphous solid; UV (MeOH) λ_{max} 211 nm (ϵ 28 000); IR neat (NaCl) ν_{max} 3372, 1610, 1597, 1422, 1160, 1051, 903, 822 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data ($CDCl_3$), see Table 1; HREIMS m/z [M^+] 308.0150 (calcd for $C_{13}H_{15}{}^{35}Cl_3O_2$, 308.0138).

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