Puupehenone-Related Metabolites from Two Hawaiian Sponges, *Hyrtios* spp.[1,2]

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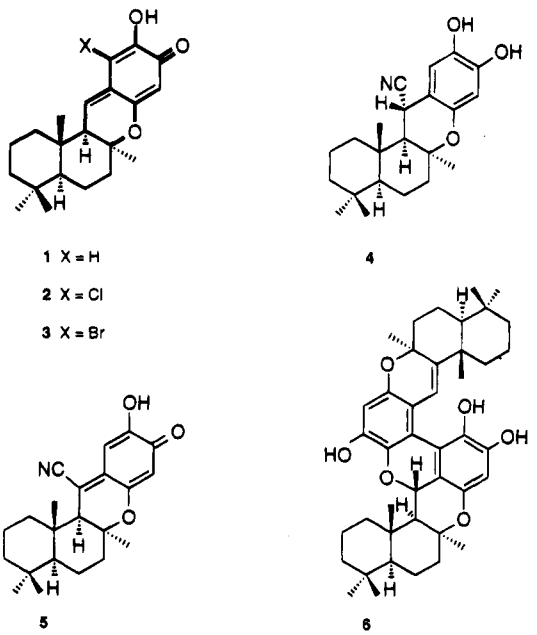
Received May 15, 1995[⊗]

In addition to several known compounds related to puupehenone (**1**), three new congeners, including a ring-contracted variant, are described. One of these, 15-oxopuupehenol, shows significant differential antitumor and antimalarial activity.

Puupehenone (**1**) and its 21-chloro and bromo derivatives (**2, 3**)[5] are representative of a distinctive family of sponge metabolites—a sesquiterpene joined to a C_6-shikimate moiety—first exemplified by the quinol–quinone pair of avarol and avarone.[6] Among the varied bioactivities which have been reported for this diverse class of compounds is the property of ilimaquinone to inhibit replication of the HIV virus.[7] We recently reported the isolation from an apparent Verongid sponge 15-cyanopuupehenol (**4**),[8] presumably arising from 1,6-addition of HCN to puupehenone (**1**). From the same Verongid sponge, we subsequently isolated[9] 15-cyanopuupehenone (**5**) and an unsymmetrical dimer, dipuupehetriol (**6**), in addition to the major constituent, puupehenone (**1**). Occurrence of puupehenone and congeners from presumably Verongid sponges was contrary to a well-established chemotaxonomic pattern.[10]

We again encountered puupehenone (**1**) as the major constituent in each of two Dictyoceratid sponges, believed to be undescribed species of *Hyrtios*, collected in underwater lava tubes on the north and west shores of O'ahu at Shark's Cove and Pokai Point (July, 1991) and in sandy substrates between Molokini Crater and Maui in the Alalakeiki Channel (June, 1991). From both sponges we also isolated the 21-halo derivatives **2** and **3**; the Maui specimen also contained the previously described bispuupehenone (**7**).[11] Three new compounds were recovered from these two sponges: 21-chloropuupehenol (**8**) from the O'ahu collection; 15-oxopuupehenol (**9**) from both animals; and the ring-contracted molokinenone (**10**) from the Maui sponge.



Puupehenone (**1**) was the major constituent in both sponges. It was isolated as a yellow glass and readily identified by its NMR spectral characteristics, particularly four methyl singlets and an olefinic doublet (H15) coupled to an alicyclic proton (H9), and subsequent comparison with an authentic sample. In similar fashion the 21-halopuupehenones **2** and **3** and dimeric **7** were identified by comparison with authentic samples.[5,11]

21-Chloropuupehenol (**8**) was isolated from the O'ahu sponge as a red glass. HREIMS data pointed to a composition of $C_{21}H_{27}ClO_3$, isomeric with chloropuupehenone (**2**), but lacking a signal for H9 and displaying signals at δ 5.37 and 5.04 for two exchangeable protons. The suspected catechol system formally derivable from **2** by isomerization was fully confirmed by HMQC,

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.

(1) In part from the Ph.D. Dissertation of M. T. H. University of Hawaii, 1992.

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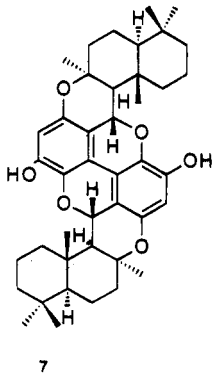

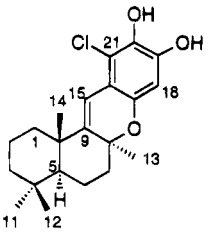

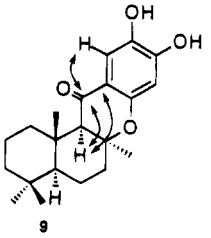

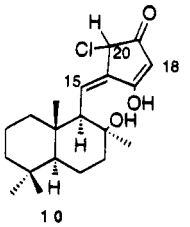

HMBC, and ROESY experiments. Me13 and Me14 (δ 25.34, 24.86) were correlated by HMBC to C9 (δ 151.78). Similarly, H15 (δ 6.30) was correlated by HMBC experiments to C8 (δ 77.00), C10 (δ 38.87), C17 (δ 146.21), and C21 (δ 114.87). The single aromatic proton H18 displayed all two- and three-bond HMBC correlations, placing chlorine at C21 (δ 114.87), *para* to H18. The two catechol carbons, C19 (δ 143.84) and C20, (δ 133.49) were assigned by comparison with calculated chemical shift values.[12]

15-Oxopuupehenol (**9**), present in both sponges, was isolated as a colorless glass; its composition, $C_{21}H_{28}O_4$, differs from that of puupehenone (**1**) by an additional oxygen atom. The new carbonyl signal (δ 194.30) was unambiguously placed by the HMBC correlations shown by arrows in the structural formula. Two broad signals of the catechol protons were seen at δ 6.60 and 6.29. The compound can be envisaged as an 1,6-adduct of water, followed by oxidation.

The final compound, molokinenone (**10**), was found only in the sponge collected near Molokini crater. It is a tan-colored glass of composition $C_{20}H_{27}ClO_3$, one carbon less than 21-chloropuupehenone (**2**). Tell-tale ^{1}H NMR signals, an H15 doublet at δ 6.28 coupled to the H9 doublet at δ 2.00, suggested a structural relationship to puupehenone (**1**) and pointed to a ring contraction to account for the missing carbon atom. The familiar H9 and H15 signals were crucial for deducing the cyclopentenone structure. C16 could be correlated to H9, H18, and H20, and H15 to C17 and C20; in addition, an NOE was observed between H15 and H20. The remaining carbons C18 and C19 were placed on the basis of their chemical shifts, δ 105.15 and 202.36. Relative stereochemistry followed from NOE measurements.

Other ring-contracted metabolites have been reported from *Dactylospongia* spp., a genus in the same family (Thorectidae) as *Hyrtios*.[13,14] In one of the cases, the dactylospongenones,[13] C21 is retained as a carboxyl on the cyclopentenone. *In vitro* degradation of hydroxyquinones to cyclopentanedione in alkali or on standing has long been observed.[15] Whether the parallel transformations take place *in vivo* or during workup is not known.

Comparison of the biological activity[16] of bispuupehenone (**7**), 15-oxopuupehenol (**9**), and molokinenone (**10**) (Table 3) shows significant differential antitumor and antimalarial activity for 15-oxopuupehenol (**9**). 21-Chloropuupehenol (**8**) decomposed before it could be examined for biological activity.

Table 1. ^{1}H NMR Data of Compounds 8–10

	8		9		10	
	ppm	*J* (Hz)	ppm	*J* (Hz)	ppm	*J* (Hz)
1	2.10 m		2.21 dt	14.5, 3.2	2.24 dt	14.8, 3.1
	1.35 m		1.67 m		1.74 d	13.3
2	1.65 m		1.70 m		1.55 m	
	1.57 m		1.62 m		1.39 m	
3	1.45 m		1.41 m		1.69 dt	14.4, 5.3
	1.15 m		1.17 m		1.12 dt	12.8, 3.5
4						
5	1.39 m		0.91 m		0.97 dd	11.5, 2.4
6	1.84 m		1.52 m		1.24 t (2H)	7.0
	1.73 m		1.41 m			
7	1.98 dd	12.8, 9.4	1.53 m		1.40 m	
	1.56 m		1.22 m		1.19 dt	13.1, 4.4
8					2.70 s (OH)	
9			1.88 s		2.00 d	6.6
10						
11	0.95 s (3H)		0.92 s (3H)		0.96 s (3H)	
12	0.86 s (3H)		0.84 s (3H)		0.85 s (3H)	
13	1.22 s (3H)		1.23 s (3H)		1.28 s (3H)	
14	1.33 s (3H)		0.85 s (3H)		0.80 s (3H)	
15	6.30 s				6.28 d	6.5
16						
17					2.70 s (OH)	
18	6.41 s		6.42 s		5.36 s	
19	5.37 s (OH)		6.60 s (OH)			
20	5.04 s (OH)		6.29 s (OH)		4.62 s	
21			7.47 s			

Table 2. ^{13}C NMR Data of Compounds 8–10

carbon	8, ppm	9, ppm	10, ppm	carbon	8, ppm	9, ppm	10, ppm
1	39.1	40.1	39.6	12	21.2	22.0	22.0
2	18.9	18.4	18.0	13	25.3	26.6	28.9
3	42.0	41.6	41.7	14	24.9	15.2	14.4
4	33.8	33.4	33.2	15	110.6	194.3	123.6
5	43.9	54.3	54.1	16	116.6	115.1	135.1
6	17.3	18.2	18.3	17	146.2	156.8	178.2
7	30.8	39.9	39.3	18	102.9	103.7	105.2
8	77.0	80.4	81.8	19	143.8	152.7	202.4
9	151.8	64.5	53.7	20	133.5	138.0	70.9
10	38.9	38.5	40.2	21	114.9	110.8	
11	32.7	33.8	33.7				

Experimental Section

Collection and Extraction. The Maui sample (2.1 kg) was collected by SCUBA from flat sandy areas (−120 ft)

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(17) The sample collected from Maui formed an incompressible, extremely brittle mat with basically incorporated calcareous debris. The surface is very deeply honey-combed, formed by the interconnection of coarse blunt primary conules with secondary fibers. The fibers and ectosome are heavily charged with spicule and sand debris. The sponge was dark brown in life and in ethanol preservative, the conules and armored surface have a whitish sheen. The O'ahu sample is very common and can be found growing on the walls and ceilings of dimly lit caves and lava tubes. The sponge is yellow in life and turns light gray in ethanol as the yellow pigment is extracted. Voucher specimens have been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, FL (Catalog No. 003-597 for O'ahu sample and 003-884 for Maui sample).

Table 3. *In Vitro* Biological Activity of Compounds 7, 9, and 10

assay (MIC, μg/mL)	7	9	10
cytotoxicity, μg/mL			
Vero	NT	3.4	NT
KB	>10	10(+3)	NT
LOVO	10(+2)	5(+2)	NT
P-388, IC_{50}	>20	1	5
A-549, IC_{50}	>20	0.5	10
HT-29, IC_{50}	>20	2	10
CV-1, IC_{50}	>20	1	NT
Topo II, IC_{50}	NT	1	NT
immunomodulator	NT		
MLR[a] (IM activity; IC_{50})		2.8	24
LCV[b] (cytotoxicity; IC_{50})		>50	>50
potency (LCV/MLR) (>25)		>18	>2
antiviral, μg/mL (% reduction)			NT
Mv 1 Lu/HSV II	>10	>10	
CV-1/HSV-1	>40	>10	
BHK/VSV	>40	<10	
antimalarial, μg/mL, IC_{50}	NT		NT
Plasmodium falciparum (D6 clone)		2.0	
Plasmodium falciparum (W2 clone)		1.3	

[a] MLR: mixed lymphocyte reaction. [b] LCV: lymphocyte viability.

between the island of Maui and Molokini crater. The O'ahu organism (300 g) was first collected by SCUBA from the lava tubes (−30 ft) on the north and west shores of O'ahu.[17] A crude lipophilic extract of each indicated the presence puupehenone as its major metabolite. The samples were exhaustively extracted with EtOH and CH_2Cl_2. The puupehenones were purified by flash chromatography with silica gel (hexane, EtOAc, MeOH; the puupehenones were eluted with the EtOAc fraction). RP-18 flash chromatography of the EtOAc fraction with MeOH/H_2O 1:1, MeOH, EtOAc eluted the puupehenone mixture in the MeOH fraction. Final purification was accomplished by HPLC using silica gel RP-18, (MeOH/H_2O 85:15).

21-Chloropuupehenol (8) was isolated (2 mg, 7.0×10^{-4}%) from the EtOAc fraction of a silica gel flash column and purified by HPLC on silica gel RP-18 (MeOH/H_2O (85:15)). Physical properties: $[\alpha]_D$ +112° (*c* 0.35, MeOH); IR neat (NaCl) 3300 (s, br), 2926 (m, br),1655 (s), 1599 (s), 1540 (s), 1458 (s), 1372 (s), 1209 (s), 1135 (m) cm^{-1}; EIMS *m/z* (fragment, %) 364.2 (M^+ + 2, 4), 362.1653 (M^+, 10) (calcd for $C_{21}H_{27}ClO_3$ 362.1649), 348.1 (23), 349.1 (35), 347.1 (100), 313.1 (5), 224.0 (10), 173.0 (7), 131.0 (10), 81.0 (9); UV (MeOH) λ_{max} 202 (14215), 224sh (6790), 248 (6850), 332 (3050), 368 sh (1985), 458 (300) nm.

15-Oxopuupehenol (9) was isolated (2 mg from the O'ahu species, 7.0×10^{-4}%; 150 mg from the Maui species, 7.0×10^{-3}%) from the EtOAc fraction of a silica gel flash column purified by HPLC on silica gel RP-18 (MeOH/H_2O 85:15). This compound is slightly more polar than the major metabolite (puupehenone) and is eluted just ahead of puupehenone and chloropuupehenone on RP-18 HPLC. Physical properties: $[\alpha]_D$ −106° (*c* 0.52, MeOH); IR neat (NaCl) 3313 (s, br), 2947 (m, br), 1740 (s), 1664 (s), 1592 (s), 1464 (m), 1389 (s), 1294 (m), 1165 (m) cm^{-1}; EIMS *m/z* (fragment, %): 346.2 (M^+ + 2, 0.5), 344.1980 (M^+, 6) (calcd for $C_{21}H_{28}O_4$ 344.1988), 334.2 (4), 316.2 (11), 288.2 (5), 219.0 (12), 193.0 (83), 136.1 (81), 109.1 (35), 95.1 (58), 81.1 (100); UV (MeOH) λ_{max} 212 (10870), 246 (6040), 284 (5595), 354 (3060) nm.

Molokinenone (10) was isolated (2 mg, 9.5×10^{-5}%) from the EtOAc fraction of a silica gel flash column and purified by RP-18 HPLC with MeCN/H_2O (70:30). $[\alpha]_D$ −400° (*c* 0.012, MeOH); IR neat (NaCl) 3380 (s, br), 2960 (s), 1700 (s), 1575 (s), 1420 (m), 1080 (m) cm^{-1}; EIMS *m/z* (fragment, %): 351.0 (M + 1, 3), 350.1670 (M^+, 5) (calcd for $C_{20}H_{27}ClO_3$ 350.1649), 317.0 (100), 288.2 (50); UV (MeOH) λ_{max} 204 (6096), 214 (5522), 284 (12367) nm.

Acknowledgment. We thank Faith Caplan, Linda Kay Larsen, and the biologists at Pharma Mar, S.A., for bioassays; Walter Niemczura, Wesley Yoshida, and Mike Burger for spectral analyses; Pauline Fiene, Mike Severns, Toshio Ichiba, and Jay Corgiat for their help with collections; the National Science Foundation, the Sea Grant College Program, and Pharma Mar, S.A., for financial support.

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **8**−**10** and HMBC spectra of compound **10** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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