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Dipuupehedione, a Cytotoxic New Red Dimer from a New Caledonian Marine Sponge *Hyrtios sp.*

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Abstract: Dipuupehedione, a cytotoxic new red dimer of puupehenone was isolated from a New Caledonian marine sponge *Hyrtios sp.* and its structure established through spectral studies, including extensive 2 D NMR spectroscopy. Dipuupehedione is active on KB cells (IC 50 = 3 µg/ml).

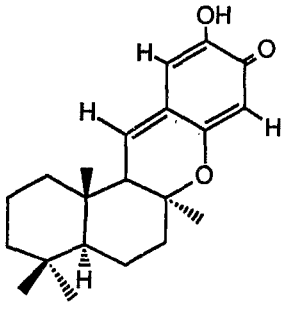
Our interest in the chemistry of marine sponges of the genus *Hyrtios* is due to the variety of compounds[1] isolated, many of which with remarkable bioactivity. Typical members are the scalarane-type sesterterpenes, represented by the well-known heteronemin[2]. As part of our chemical investigation of *Hyrtios* sponges from New Caledonia, we have previously described 12-epi-heteronemin[3] as the major compound from *Hyrtios erecta* (Keller 1889) a new member of this class of sesterterpenes. We also reported the isolation of sesterterpenes of the manoalide family: thorectolide monoacetate and thorectolide, major products[4] of a New Caledonian *Hyrtios sp.* However, from another marine sponge *Hyrtios erecta* (Keller 1889) collected in the Red Sea, we have isolated a new and unexpected β-carboline[5]. We now have studied the metabolites of another New Caledonian *Hyrtios* specimen. Interestingly, we isolated the known puupehenone, co-occuring with a red dimer, the subject of this report.

Lyophilized sponges of *Hyrtios sp.* (500 g), collected by SCUBA diving in New Caledonia (East Coast), were immersed in CH_2Cl_2 at room temperature during two days. The crude CH_2Cl_2 extract of this sponge showed significant cytotoxic activity on KB cells, antimicrobial activity against *Staphylococcus aureus*, antifungal activity against *Candida albicans*. The CH_2Cl_2 extract (7.7 g) was chromatographed on a silica gel column, eluted with hexane/AcOEt 8:2, followed by a Sephadex LH-20 gel filtration ($CHCl_3$/MeOH 2:8) and yielded puupehenone **1** (0.02% dry weight) and compound **2** (0.006% dry weight).

Puupehenone was readily identified by comparison with the spectral data reported in the literature[6].

Compound **2** was obtained as a red glassy solid. In the FAB spectrum the strongest ion was the peak at *m/z* 653. Preliminary comparison of the spectral data of **1** and **2** showed many similarities. In the ^1H NMR spectrum, signals in the aliphatic region were almost identical with those of puupehenone **1**. The main differences were the lack of two ethylenic protons and the presence of one singlet deshielded proton at δ 4.32 ppm (see Table 1). The ^{13}C NMR spectrum displayed 21 signals, suggesting that **2** should be a symmetrical dimer from **1**. High Resolution FAB mass spectrum gave for the ion at *m/z* 653 the formula $C_{42}H_{53}O_6$ (MH^+ 653.3842150, Δ mmu +3.7). Moreover, the IR spectrum revealed the absence of an OH group in **2**. Absorption maxima in the IR

spectrum at 1630, 1658, 1690 cm^{-1}, together with signals in the ^{13}C NMR spectrum at 180.1, 186.3 suggested the presence of conjugated carbonyl groups and an aromatic nucleus. A useful information was provided by the HMBC experiments, which gave correlations from the proton at δ 4.32 ppm to the three aromatic signals and from the ethylenic proton at δ 6.03 ppm (108.8ppm) to the signals at 186.3, 130.8 and 166.9 ppm. Some other correlations in the long-range 2D spectrum are reported in Fig.1. Owing to the dimeric and symmetrical structure of **2**, the signals at 130.8, 133.5 and 136.4 ppm can therefore be attributed to half of an aromatic nucleus, which was considered as the backbone of the molecule. Taking into account the above data for 17 degrees of unsaturation of the dimer, the structure of compound 2 was deduced as depicted below. The peak at *m/z* 653 corresponded to the $[MH^+ + 2]$ ion, characteristic of an ortho-quinone[7]. A complete assignment of all the protonated carbon resonances was unambiguously obtained thanks to the HMQC spectrum, except for the C-6 chemical shift. The ^1H-^1H COSY spectrum of **2** revealed a correlation from H-5 at δ 1.09 ppm to H-6 at δ 1.64 ppm, which fixed the C-6 chemical shift at δ 18.1 ppm.



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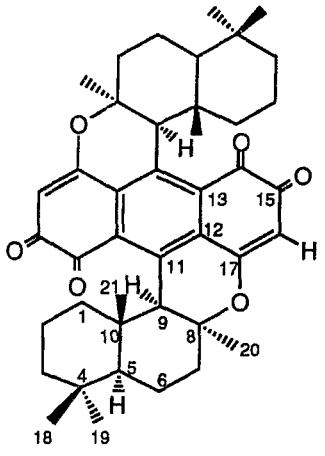


2

The configuration was assigned from interpretations of ^1H NMR spectrum and difference nuclear Overhauser experiments. The CH_3-20 signal at δ 14.6 ppm must be assigned to an axial methyl group at a *trans* ring junction and the CH_3-20 signal at δ 28.8 ppm be located at a *cis* ring junction[7]. Furthermore, irradiation of the signal at δ 0.56 ppm (CH_3-21) produced an enhancement of the signal at δ 0.79 ppm (CH_3-18, 3%), which bolstered the above mentioned relative configuration assignments. Irradiation of the signal at δ 4.32 ppm produced an enhancement in the signal at δ 1.34 ppm (CH_3-20, 1.6%) and at δ 1.09 ppm (CH-11, 3.6%), corroborating the *cis* BC junction.

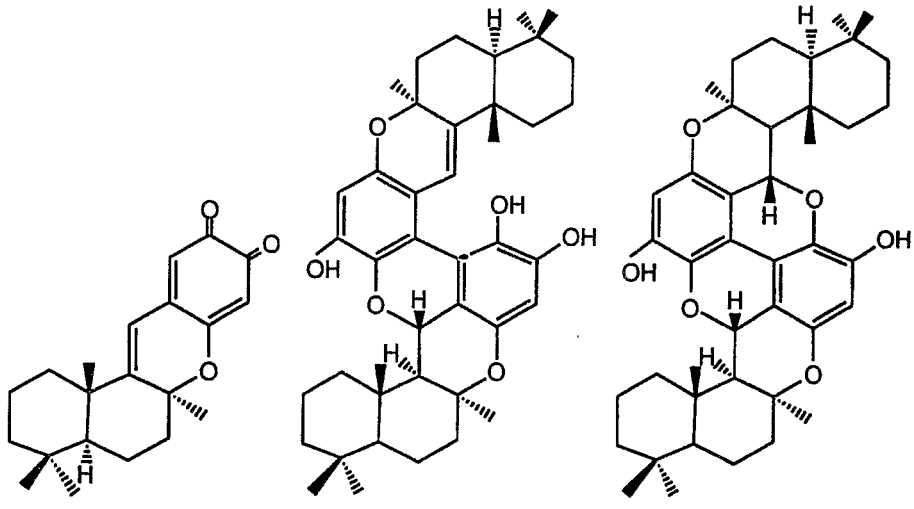
Compound **2** corresponds probably to the amorphous red pigment, formula $C_{42}H_{52}O_6$, already mentioned but not identified during isolation of puupehenone from a yellow incrusting sponge, collected off the Hawaiian islands of Lanai and Oahu[7] and later identified as a *Heteronema sp.* Compound **2** appeared to be the dimer of the ortho-quinone, puupehedione **3** previously isolated besides an asymmetrical dimer: dipuupehetriol **4** from an unidentified Verongid sponge (family Aplysinellidae)[8]. The sole symmetrical dimer already reported in the literature is a colorless compound, bispuupehenone **5** isolated from a Tahitian sponge *Hyrtios eubamma*[9] (Laubenfels 1954). These puupehenone-related metabolites exhibit a variety of biological activities (cytotoxic,

antiviral, antifungal immunomodulatory) to varying degrees: if bispuupehenone **5** showed no cytotoxic activity, puupehedione **3** exhibited cytotoxic and potential immunomodulatory activity and dipuupehetriol **4** showed distinct selectivity against A-549 (human lung cancer line) and antiviral CV-1 activities[8].

Table 1: ^{13}C (75.45 MHz, δ in ppm) and ^{1}H NMR (300.13 MHz, δ in ppm, (mult.), *J* Hz) chemical shift assignments of compound **1** and compound **2**.

Carbon n°	Compound 1		Compound 2	
	^{1}H $CDCl_3$	^{13}C $CDCl_3$	^{13}C $CDCl_3$	^{1}H $CDCl_3$
1	1.61(m) - 1.08(m)	39.7	41.1	1.45 (m) - 1.10 (m)
2	1.48 (m)	18.2*	18.0	1.29 (m)
3	1.41 (m) - 1.13 (m)	41.4	38.4	1.04 (m) - 0.76 (m)
4		33.1	33.4	
5	0.86 (m)	53.5	53.7	1.09 (m)
6	1.33 (m)	17.9*	18.1	1.64 (m)
7	2.08 (dd, 11.3, 2.6) - 1.47 (m)	38.9	38.9	2.28 (dd, 14, 2.8) - 1.72 (m)
8		78.8	81.0	
9	1.98 (d, 7)	54.6	49.1	4.32 (s)
10		41.9	41.0	
11	6.61 (dd, 1, 7)	141.2	136.4*	
12		129.0	130.8	
13	6.15 (s)	105.6	133.5*	
14		147.4	186.3	
15		182.2	180.1	
16	5.78 (d, 1)	105.9	108.8	6.03 (s)
17		162.8	166.9	
18	0.76 (s)	21.7	21.8	0.79 (s)
19	0.83 (s)	33.5	33.6	0.90 (s)
20	1.14 (s)	27.7	28.8	1.34 (s)
21	0.73 (s)	14.9	14.6	0.56 (s)
OH	6.67 (brs)			

*: may be inversed with the closest values.



3 **4** **5**

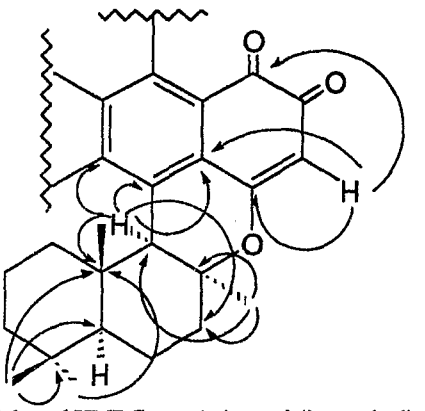


Fig. 1: Selected HMBC correlations of dipuupehedione **1**.

The crude extract possessed potent antimicrobial and antifungal activities due to the presence of puupehenone, which exhibited similar antibiotic and antifungal activity with 12 mm inhibition at 50 µg/disk against *Staphylococcus aureus* and *Candida tropicalis*, and strong cytotoxic activity on KB cells (EC_{50} = 0.8 µg/ml). In contrast, dipuupehedione **2** showed no antibiotic activity on *Staphylococcus aureus*, no antifungal activity on *Candida tropicalis* but cytotoxic activity on KB cells (EC_{50} = 3 µg/ml). Earlier biological investigations of puupehenone **1** also mentioned cytotoxic activity against human lung (A549), human colon (HCT-8) and human mammary (MCF-7) cancer cell lines and against P388 mouse leukemia[10].

It seems likely that puupehenone can dimerize by oxidation in a number of different forms, some biologically active and some completely inactive. The large distribution of puupehenone and relative compounds illustrates the scope of help of chemical analysis for reinvestigating classification of sponges based on morphological analysis.

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