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Full Paper

Ilimaquinone and 5-*epi*-Ilimaquinone: Beyond a Simple Diastereomeric Ratio, Biosynthetic Considerations from NMR-Based Analysis

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Dactylospongia metachromia and *Dactylospongia elegans* collected from French Polynesia were studied with a particular focus on the variation of the diastereomeric ratio between ilimaquinone (**4**) and 5-*epi*-ilimaquinone (**5**). More than 100 samples, covering an area of 4100 km^2, were studied to try to clarify this intriguing issue. Nuclear magnetic resonance appeared as the non-destructive, straightforward technique of choice for a relative quantitative study. A random distribution, unique at that point in nature, is observed and leads to biosynthetic considerations. Biological evaluation of both compounds was also performed and showed moderate discrepancies in cytotoxicity and apoptosis induction.

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Introduction

Sesquiterpenic *para*-quinones constitute a homogeneous family of natural substances of marine origin mainly found in sponges from the order Dictyoceratida[1,2] that display valuable biological properties.[3] Selected examples of such sesquiterpene quinones (Fig. 1) bearing a clerodane decalin – differing from the *endo* ($\Delta_{3,4}$) or *exo* ($\Delta_{4,11}$) position of the double bond – include avarone (**1**, monosubstituted quinone), and C-18- and C-19-methoxyavarone (**2** and **3**, disubstituted quinones).[1,2] Of particular interest to us are trisubstituted quinones such as ilimaquinone (**4**),[4] 5-*epi*-ilimaquinone (**5**),[5] recently described 5,8-di-*epi*-ilimaquinone (**6**),[6] or isospongiaquinone (**7**)[7] that differ from the configuration at carbons 5 (*trans*- or *cis*-clerodane skeleton) and 8 and/or the position (*endo* versus *exo*) of the decalin double bond. Because of a highly interesting and unique biological profile (especially the ability of ilimaquinone to reversibly dislocate the Golgi apparatus of living cells)[8] and its abundance within specific marine organisms, ilimaquinone (**4**) and congeners attracted our attention, particularly in relation with the above-mentioned stereochemical particularities distinguishing compounds **4**–**7**. As part of a program aiming at the sustainable valorization of marine biodiversity from French Polynesia, we wish to disclose in this paper the first study dedicated to better understanding the co-existence of these sesquiterpenic quinones in two different *Dactylospongia* species (*D. metachromia*[9] and *D. elegans*[10]). Additionally, biosynthetic considerations as well as consequences on the chemical and biological properties of **4** and **5** are provided in this work. The particular point of the 'real' origin of these quinonic sesquiterpenes (i.e. sponges versus endosymbionts), even if not known concerning **4** and close congeners, was clarified earlier in *Lamellodysidea herbacea* (earlier found as *Dysidea herbacea*, Dysideidae) and *Dysidea avara* (Dysideidae) by Faulkner and Unson[11] and Uriz et al.,[12] respectively.

Results and Discussion

*Ilimaquinone versus 5-*epi*-Ilimaquinone Diastereomeric Ratio*

D. metachromia samples were collected from 10 different locations in French Polynesia (Fig. 2) covering three archipelagos (Tetiaroa atoll from Société, Tikehau, Rangiroa, Toau, Takaroa, Fakarava, Hereheretue, Makemo, and Anuanuraro from Tuamotu, and finally three sites in Gambier archipelagos), thus corresponding to a covered area of ~4100 km^2.

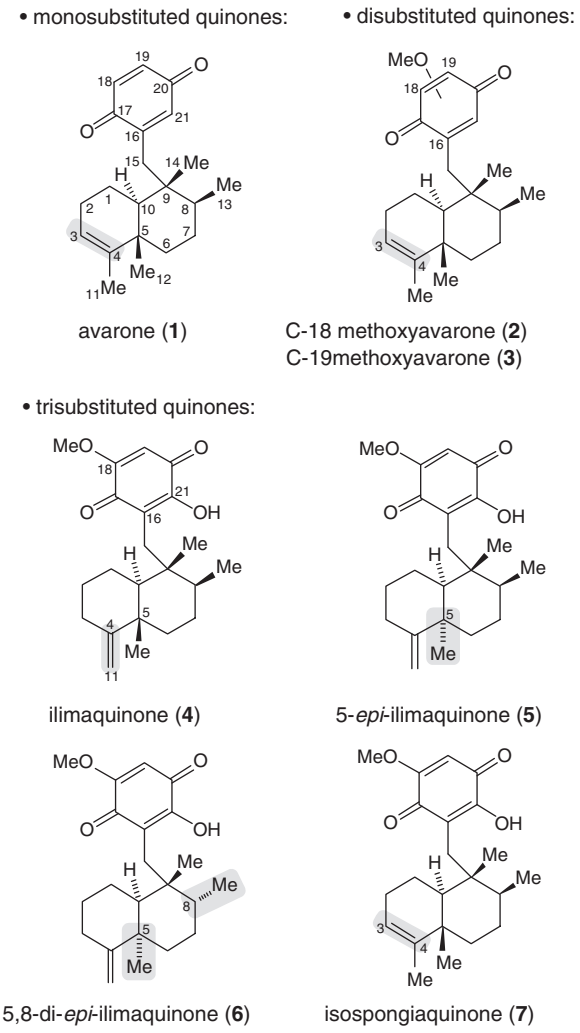


Fig. 1. Selected examples of sesquiterpene quinones of marine origin. The grey boxes highlight the differences in the sesquiterpene moiety.

Two specimen collected in Fiji Islands were also studied. We were able to collect a unique set of over 100 samples of *D. metachromia* that could be analyzed. Ilimaquinones **4** and **5** are the most abundant compounds in the total methanolic extract on HPLC chromatograms in *D. metachromia.* Ilimaquinone (**4**)/5-*epi*-ilimaquinone (**5**) diastereomeric ratio (dr) could be evaluated rapidly by ^{1}H NMR analysis of ethylic ether crude extracts of the sponges (integration of the C-11 *exo*-methylene singlets CH_2-11 **4**: 4.40, 4.42 ppm; **5**: 4.66, 4.69 ppm in $CDCl_3$). Data analysis clearly shows discrepancies between locations and also small variations between specimens from the same location at different depths. From 100 % ilimaquinone in specimens from Anuanuraro island to ~90 % 5-*epi*-ilimaquinone in specimens from Gambier Islands (Tables 1 and 2), the **4**/**5** ratio varies in an unpredictably manner.[13,14]

Biosynthetic Considerations

Puzzled by these observations, we turned our attention to the biosynthetic hypotheses (Scheme 1) of this family of natural substances; the biosynthesis process may start with the farnesylation of an aromatic ring (the precursor of the quinone moiety). A plausible biosynthetic pathway involves initial folding of the suitable precursor **8** within the active site of a specific terpene cyclase.[15] The successive formation of two carbocationic intermediates **9** and **10** may then proceed, resulting from *peri*-planar Wagner–Meerwein hydrogen and methyl shifts. At the last stage from **10**, two pathways (1 or 2) may finally explain the outcome towards formation of ilimaquinone (**4**) versus 5-*epi*-ilimaquinone (**5**) before termination of the cascade via a proton loss of carbocations **11** and **12**, respectively. At this stage, aside from a spontaneous epimerization at carbon 5 (never observed experimentally), at least two distinct explanations may arise: (i) a variable expression of two distinct terpene cyclases affording **4** or **5**; (ii) a lack of selectivity within the active site of a single terpene cyclase that may be correlated to some extent to a certain degree of liberty, especially of intermediate of type **10** that randomly affords both compounds.[16] To the best of our knowledge, the present study is the first report of a chemical variation at a non-epimerizable carbon centre (see chemical reactivity studies below).

Furthermore, and very interestingly, the antipodal carbocationic intermediate *ent*-**8** was shown, in a sample of *D. elegans* collected near Mooloolaba (Queensland, Australia), to be at the origin of another complete series of structures such as (+)-hyatellaquinone (**13**, Scheme 2).[17] In our case, *D. elegans* samples from Marquesas archipelago revealed the presence of either ilimaquinone (**4**, Nuku Hiva island) or isospongiaquinone (**7**, Tahuata and Fatu Hiva islands, Scheme 3) as the main compounds, the latter **7** being described for the first time in this species.

Chemical Transformations

Both **4** and **5** could not be individually resolved on classic silica gel TLC plate or by silica gel preparative flash chromatography. Fortunately, they could be separated using silver nitrate-impregnated silica gel with CH_2Cl_2 and methanol (MeOH) as the eluents.[17] In order to experimentally counteract the arguments of a possible spontaneous epimerization in the course of extraction, the reactivity of ilimaquinone was investigated and the latter was found to be stable under extraction conditions and prolonged contact with silica gel. Under acidic conditions (Scheme 4) and following protonation of the exocyclic double bond, both **4** and **5** provided neomamanuthaquinone (**15**)[18] after Wagner–Meerwein-type rearrangements following pathway 1 and isospongiaquinone (**7**)[7] and 5-*epi*-isospongiaquinone (**14**),[19] respectively, according to pathway 2. The formation of **15** in similar conversion yields and similar ratios to those obtained for **7** and **14** demonstrates that both the C-5 β-methyl of **4** and the C-5 α-methyl of **5** can undergo Wagner–Meerwein migration without interconversion. These results confirm that **4** and **5** are not interconverted during the extraction process.

Biological Evaluation

Despite a large amount of biological data available for ilimaquinone, a lack of comparative data between **4** and **5** prompted us to evaluate both compounds. To further characterize their cytotoxicity profiles, we investigated their effects on the proliferation of four human tumour cell lines: cervical adenocarcinoma (HeLa), prostate adenocarcinoma (PC3), and uterine sarcoma (MES-SA) and its multidrug-resistant subline (MES-SA/Dx5). The results presented in Table 3 revealed that both **4** and **5** displayed more potent cancer cell growth inhibitory activities against MES-SA. Overall, ilimaquinone **4** was moderately more cytotoxic than *epi*-ilimaquinone **5**. Finally, they

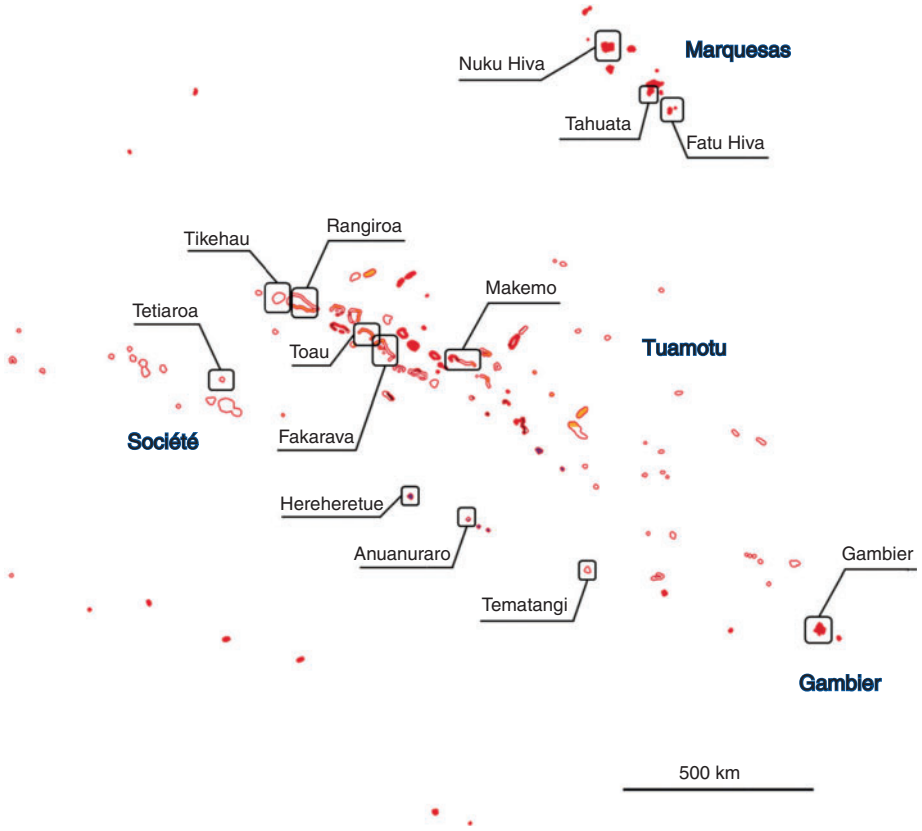


Fig. 2. Map of sample collection sites in French Polynesia.

Table 1. Diasteromeric ratio[A] between ilimaquinone (4) and 5-*epi*-ilimaquinone (5) from samples of *D. metachromia* collected from different areas of French Polynesia and Fiji

Location	Number of samples	% of **4**/**5** ratio mean value (standard deviation)[B]
Tetiaroa	15	61.5/38.5 (±6.2)
Tikehau	18	77.5/22.5 (±5.2)
Rangiroa	10	76.5/23.5 (±2.1)
Toau	8	100/0[C]
Fakarava	16	87.5/12.5 (±10.8)
Makemo	1	85/15
Hereheretue	5	100/0[C]
Anuanuraro	4	100/0[C]
Tematangi	19	43.5/56.5 (±6)
Gambier	7	10.5/89.5 (±1.7)
Ngele Levu (Fiji)	1	100/0[C]
Vanua Levu (Fiji)	1	100/0

[A]Determined by ^{1}H NMR spectroscopy ($CDCl_3$, 300 MHz) analysis of a crude ethylic ether extract of frozen dried sponges and integration of C-11 methylene singlets.
[B]Mean values of ratios and standard deviations are provided for information for each collecting spot.
[C]NMR spectra were recorded on ~50–100 mg/0.5 mL $CDCl_3$; 100/0 ratios were obtained when ^{1}H-signals of **5** could not be detected from the baseline.

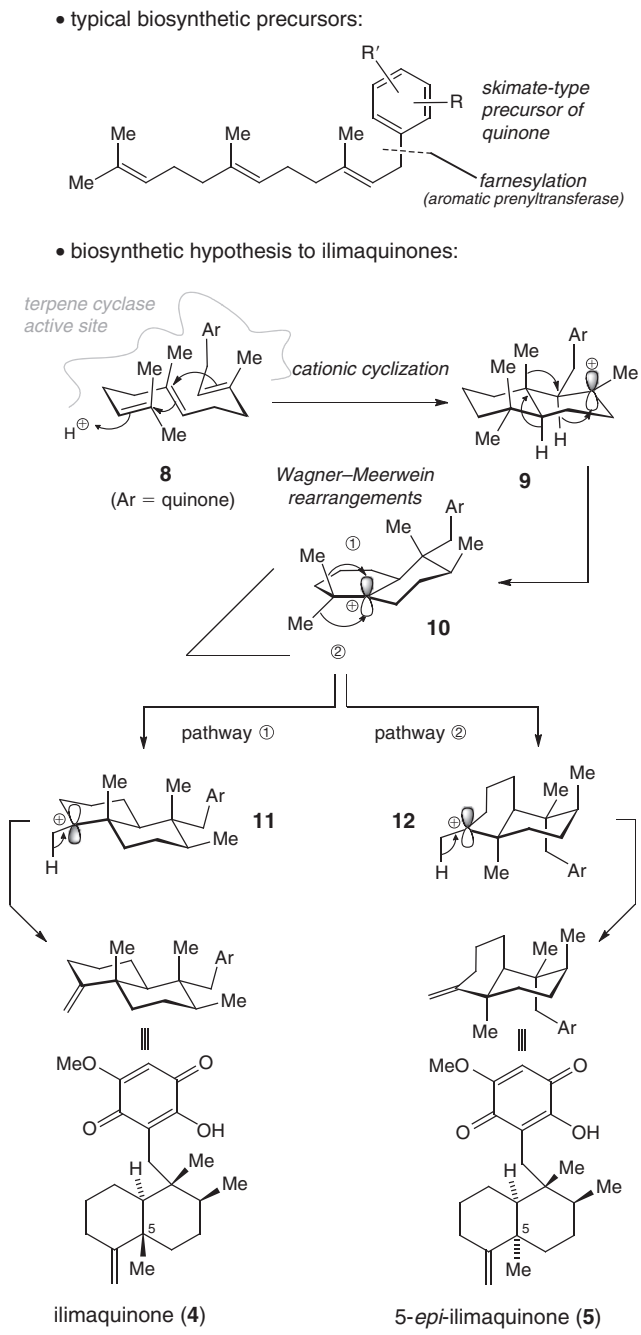
Table 2. Ilimaquinone (4)/5-*epi*-ilimaquinone (5) diastereomeric ratio[A] of *D. metachromia* collected at different depth in two areas at Rangiroa atoll (Tiputa pass and Avatoru pass)

Depth of collection (m)	Tiputa pass[B]	Avatoru pass[C]
7	76/24; 79/21[D]	87/13; 76/24[D]
17	75/25; 75/25[D])	84/16; 75/25[D]
27	83/17; 64/36[D]	86/14; 79/21[D]
37	83/17; 41/59[D]	89/11; 89/11[D]
47	–	74/26[E]
57	–	89/11; 70/30

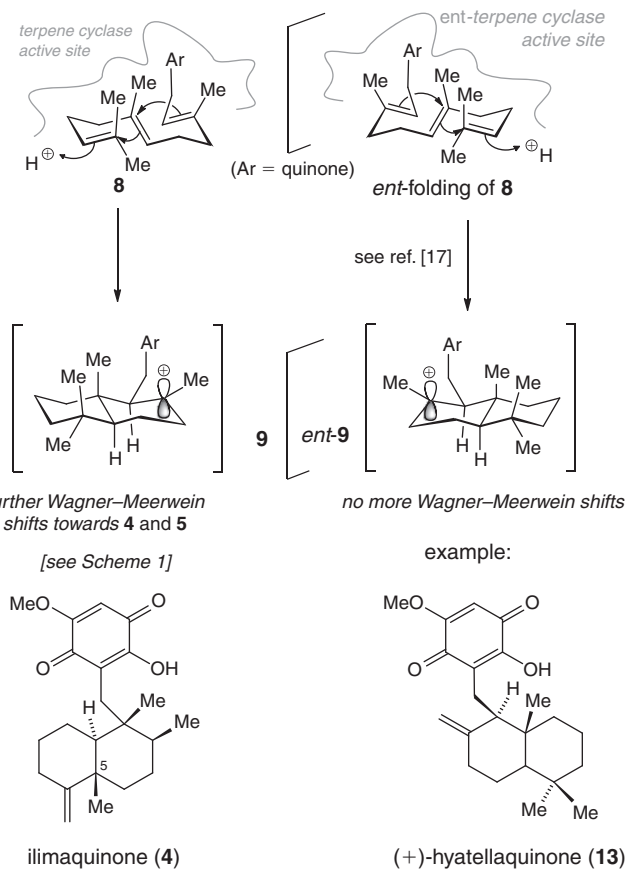
[A]Determined by ^{1}H NMR spectroscopy ($CDCl_3$, 300 MHz) analysis of a crude ethylic ether extract of lyophilized sponges and integration of C-11 methylene singlets.
[B]14°58.209 S; 147°37.375 W.
[C]14°56.054 S; 147°42.492 W.
[D]Two samples were collected.
[E]One sample was collected.

were both found inactive against PC3 and HeLa cells (half-maximal inhibitory concentration (IC_{50}) > 50 µM). To verify a presumed correlation between the cytotoxicity of **4** and **5** and their ability to induce apoptosis, the activity of caspase-3 and caspase-7 was measured by a specific apoptosis assay on the most sensitive cell lines. Cleavage of pro-caspases to active caspases is one of the hallmarks of apoptosis. MES-SA and MES-SA/Dx5 cells were incubated with 50 and 5 µM of both **4** and **5** (concentrations ranging between ½IC_{50} to 2IC_{50} of the two compounds) for 24 h, and the activities of caspase-3 and caspase-7 were evaluated using the standard caspase assays. The results depicted in Fig. 3 show a significant dose-dependent increase in proteolytic activity of caspases in both MES-SA and MES-SA/Dx5 cell lines.

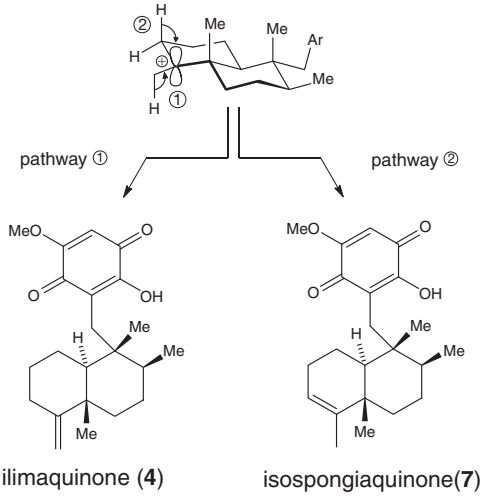
These data suggested that **4** and **5**, in addition to their cytotoxic properties, also induced apoptosis in the above-mentioned cell lines. Again, ilimaquinone was found to be more



Scheme 1. Proposed biosynthetic pathways of ilimaquinone (**4**) and 5-*epi*-ilimaquinone (**5**).



Scheme 2. Antipodal cyclization processes observed in *Dactylospongia* sp.: ilimaquinone versus hyatellaquinone series.

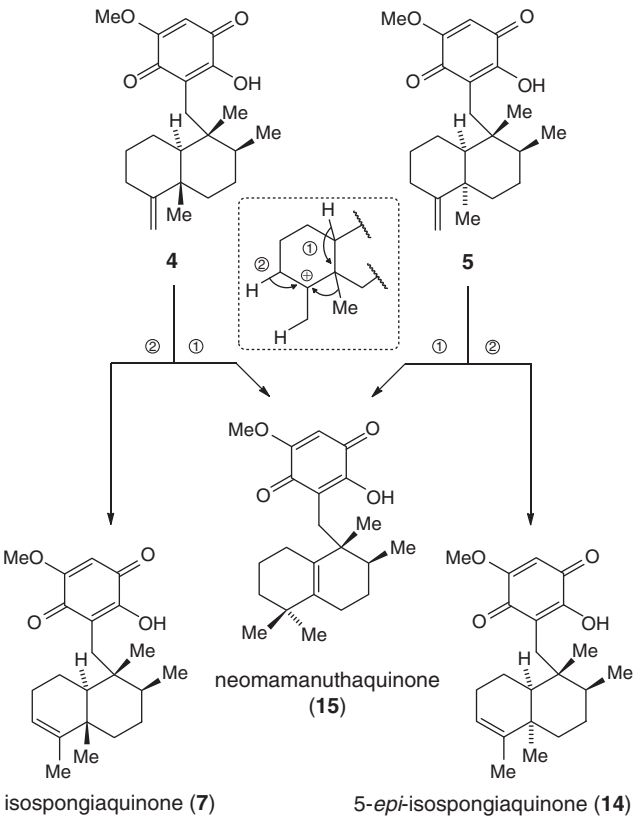


Scheme 3. Pathways for the formation of ilimaquinone (**4**) and isospongiaquinone (**7**) in *D. elegans*.

potent in inducing apoptosis than its epimer. Finally, it is interesting to point that the potency of ilimaquinone and its epimer to induce apoptosis at 50 μM was more important for the multidrug-resistant cell line MES-SA/Dx5.

Conclusions

A striking random fluctuation in the ratio between ilimaquinone and its C-5 epimer has been studied in one of the largest collections of samples (both in terms of number of specimens and the geographical scope of the study) for a single species of marine sponge. Slight differences in the biological properties of both compounds have been revealed. Biosynthetic hypotheses have been put forward and merit further investigations. From one region to another, the **4**/**5** diastereomeric ratio varies significantly in an unpredictable manner from 100/0 to 10/90. This variation may originate from either the lack of selectivity of a terpene cyclase or the presence of two different iso-enzymes. The genomic study confirmed the identity of a single species, and this empirical observation indicates the existence of different molecular phenotypes.



Scheme 4. Isomerization under acidic conditions. Reagents and conditions: MeOH/acetic acid/HCl, room temperature, 15 min (**7**: 48 % yield, **14**: 53 % yield, **15**: 32 % yield, 35 % yield, respectively from **4** and **5**).

Table 3. IC_{50} (μM) of compounds 4 and 5 in the presence of human cancer and normal cell lines

Values are mean ± standard errors of three independent experiments

	HeLa	MES-SA	MES-SA/Dx5	PC3
4	>50	2.44 ± 0.36	10.48 ± 1.45	>50
5	>50	2.56 ± 0.70	12.54 ± 1.45	>50

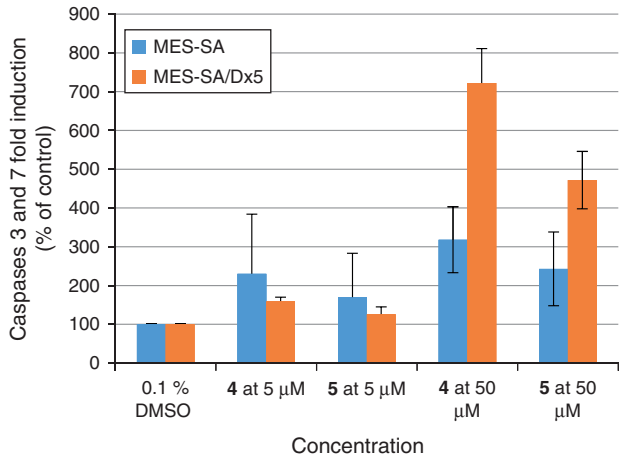


Fig. 3. Apoptotic effects of **4** and **5** on MES-SA and MES-SA/Dx5 cells. Results are expressed as the percentage of apoptotic cells detected following 24 h of treatment with **4** and **5** at two concentrations.

Experimental

General Experimental Procedures

Infrared spectra were recorded on a Vector 22 Bruker spectrometer. NMR spectra were recorded on a Bruker AM-300 (300 MHz) and a Bruker AM-400 (400 MHz) instruments using [D4]methanol, [D]chloroform and [D6]DMSO as solvents. The solvent signals were used as references. Multiplicities are described by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. High-resolution–electrospray ionization–mass spectrometry and liquid chromatography–mass spectrometry were run on a Thermoquest TLM LCQ Deca ion-trap spectrometer equipped with a sunfire analytical C_{18} column (150 mm × 2.1 mm; 3.5 μm; Waters) and a UV-visible diode array detector (190–600 nm; Waters 2996). Analytical thin layer chromatography (TLC) was performed using Merck silica gel F254 (230–400 mesh) plates, and analysis was conducted either using UV light or by staining upon heating with vanillin solution (2 g vanillin, 1 mL concentrated H_2SO_4, 100 mL ethanol). For silica gel chromatography, the flash chromatography technique was used, involving Merck silica gel 60 (230–400 mesh) and analytical silver nitrate-impregnated silica gel TLC was obtained by dipping p. a. grade TLC plates in a 2 wt-% solution of $AgNO_3$ in acetonitrile and drying at 80°C. Silver nitrate-impregnated silica gel (2 wt-%) was obtained by suspension of silica gel (100 g) in a mixture of a 2 wt-% solution of $AgNO_3$ in acetonitrile (100 mL) and dichloromethane (300 mL), followed by further drying at 40°C under reduced pressure. All the chemicals and solvents were purchased from Aldrich and SDS (France) and required no further purification.

Collection of Samples

The sponges were collected during SCUBA diving on field trips led aboard the research vessel *Alis* in 2007 (Fiji, BSMF),[20] 2009 (Marquesas, BSMPF-1),[21] 2011 (Tuamotu, Tuam'2011),[22] and in Gambier archipelago under the project 'Coralspot' in 2010. Sampling at specific depths was carried out in 2013 at Rangiroa (Tuamotu) using local facilities. See Table S2 in the Supplementary Material.

Marine Sponge Specimens

Taxonomic identifications were performed by the team of John N. A. Hooper at the Queensland Museum (Brisbane, Australia), where voucher specimens of *D. metachromia* de Laubenfels, 1954 (class Demospongiae, order Dictyoceratida, family Thorectidae) are deposited under the accessing numbers G333275 (Gambier, French Polynesia), G333295 and G333722 (Tuamotu, French Polynesia), and G324634 (Vanua Levu, Fiji). The voucher specimen of *D. elegans* Thiele, 1899 (class Demospongiae, order Dictyoceratida, family Thorectidae) is deposited under the accessing number G331081 (Marquesas, French Polynesia).

Genetic Analysis of Dactylospongia *Species*

Genetic differences of the Polynesian *D. metachromia* were tested by comparing sequence signatures of the internal transcribed spacers (ITSs) 1 and 2 of the nuclear ribosomal cistron. The ITS of multiple samples per collection site, following the biochemical analyses, was sequenced: Hereheretue, Anuanuraro, Toau, Tikehau (1 site with 6 samples each), Fakarava (2 sites with 6 samples each), Rangiroa (3 sites with 6 samples

each), Tetiaroa (2 sites with 2 samples each), and one sample of Makemo. DNA was extracted following standard methods,[23] and amplification of ITS fragments was performed with primers ITS-RA2-fwd and ITS2.2-rvse,[24] or ITS1-fwd and ITS4-rev.[25] Forward and reverse strands were sequenced with the ABI BigDye Terminator 3.1 chemistry following the manufacturers protocol on an ABI3730 capillary sequenced in the Genomic Sequencing Unit of the LMU. Sequencing processing and assembly was facilitated with CodonCodeAligner (www.codoncode.com) before analysis with *MacClade version 4.08*[26] and *Geneious version 8.1*.[27] Sequences are deposited in NCBI GenBank and the Sponge Barcoding Database (www.spongebarcoding.org). Sites suspected of displaying intragenomic polymorphisms were coded with IUPAC codes and removed from the analysis. The resulting alignment consisted of 807 characters spanning over partial 18S and 28S and complete ITS1, 5.8S, and ITS2. All samples from all locations assessed has identical ITS signatures with the exception of two specimens from Hereheretue (1.7% difference) and the one Makemo sample (1.5% difference). Due to the fact that ITS is a highly variable marker and the genetic differences can be regarded as low when compared with other dictyoceratid taxa,[28] there is no basis for interpretation as different species.

*Extraction and Purification of **4** and **5***

Freeze-dried sponge material (300 g) was extracted with a mixture of CH_2Cl_2 and MeOH (1 : 1, v/v, 1 L; 3 × 1 L) using an ultrasound cleaner bath for 1 h. Combined extracts were concentrated under reduced pressure yielding a dark purple crude extract (~100 g). The crude extract was desalted by partition between ethyl acetate (1 L) and water (3 × 250 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure yielding an organic extract (30 g). The organic extract was subjected to purification by flash chromatography over silica gel (elution gradient: petroleum ether/ethyl acetate/methanol 1 : 0 : 0 → 0 : 1 : 0 → 0 : 9 : 1), giving four fractions named F1, F2, F3, and F4. F3 (11 g, 3.6%), which was eluted with a gradient of petroleum ether/ethyl acetate 7 : 1 → 1 : 1, corresponds to a mixture of ilimaquinone **4** and 5-*epi*-ilimaquinone **5**. F3 was resolved by flash chromatography over silver nitrate-impregnated silica gel (2 wt-%) (elution gradient: petroleum ether/dichloromethane/methanol 2 : 8 : 0 → 0 : 1 : 0 → 0 : 9 : 1), affording 5-*epi*-ilimaquinone **5** (5 g, 1.6%) followed by ilimaquinone **4** (3.3 g, 1.1%). Compounds **4** and **5** displayed spectroscopic data identical to those previously reported (see Supplementary Material).

*Determination of Ilimaquinone/**5**-epi-Ilimaquinone Ratio*

Freeze-dried sponge material (500 mg) was extracted with diethyl ether (3 × 10 mL) using an ultrasound cleaner bath for 15 min. After filtration, the extracts were concentrated under reduced pressure. The diastereomeric ratio between **4** and **5** was determined by ^{1}H NMR spectroscopy analysis conducted in [D] chloroform. Signals of the methylene function of ilimaquinone (**4**) (4.40/4.42 ppm) and 5-*epi*-ilimaquinone (**5**) (4.66/4.69 ppm) were used as references.

*Acidic Isomerization of **4***

To a solution of **4** (100 mg, 0.28 mmol) in MeOH (10 mL) was added a mixture of a solution of aqueous 35% HCl (10 mL) and acetic acid (10 mL). After 15 min of stirring, the reaction mixture was quenched by addition of water (15 mL) and extraction with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduce pressure. The obtained residue was purified by flash chromatography over silver nitrate-impregnated silica gel (2% w/w, elution gradient: petroleum ether/dichloromethane 1 : 1 → 0 : 1), affording **7** (48 mg, 48%) and then **15** (32 mg, 32%).

*Acidic Isomerization of **5***

To a solution of **5** (200 mg, 0.56 mmol) in MeOH (20 mL) was added a mixture of a solution of aqueous 35% HCl (10 mL) and acetic acid (10 mL). After 15 min of stirring, the reaction mixture was quenched by addition of water (20 mL) and extraction with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduce pressure. The obtained residue was purified by flash chromatography over silver nitrate-impregnated silica gel (2 wt-%) (elution gradient: petroleum ether/dichloromethane 1 : 1 → 0 : 1), affording **14** (106 mg, 53%) and then **15** (71 mg, 35%). Compounds **7**, **14**, and **15** displayed spectroscopic data identical to those previously reported (see Supplementary Material).

Biological Evaluation

HeLa cell line was originally purchased from the American Type Culture Collection (ATCC) and maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. MES-SA cells and MES-SADX5, from ATCC, were grown in McCoy's 5A (ATCC) with 10% fetal bovine serum and 1% penicillin/streptomycin. PC-3 cell line was kindly given by O. Filhol (BCI laboratory, iRTSV, Grenoble, France) and maintained in RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. The cell viability assay was performed in 96-wells microplates. Depending of the cell type and the rate of cell growth, cells were seeded at 8000 cells per well (HeLa, PC-3) or 12 000 cells per well (MES-SA, MES-SA DX5) in 90 µL culture medium and allowed to grow for 24 h. Compounds at different concentrations were then added to the cell-containing wells. Cells were allowed to grow for additional 24 h. Cell viability was evaluated using the CellTiter-Glo® luminescent cell viability assay (Promega France) according to supplier's protocol. The results were expressed as the percentage of viable cells relative to parallel, control cell seeding (0%, 25%, 50%, 75%, and 100%). Apoptosis induction assay was performed in 96-wells microplates. Depending on the cell type and the rate of cell growth, cells were seeded at 8000 cells per well (HeLa, PC3) or 12 000 cells per well (MES-SA, MES-SA DX5) in 90 µL culture medium and allowed to grow for 24 h. Compounds at different concentrations or extracts at different dilutions were then added to the cell-containing wells. Cells were allowed to grow for additional 24 h. Apoptosis induction was evaluated using the Caspase-Glo® 3/7 assay (Promega France) according to supplier's protocol. The results were expressed as fold of apoptosis induction relative to basal apoptosis induction measured on untreated, control cells.

Supplementary Material

Full analytical description, and ^{1}H, ^{13}C NMR spectra of compounds **4**, **5**, **7**, and **14** and sample collection complementary data are available on the Journal's website.

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(c) M. T. Do, M. Na, H. G. Kim, T. Khanal, J. H. Choi, S. W. Jin, S. H. Oh, I. H. Hwang, Y. C. Chung, H. S. Kim, T. C. Jeong, H. G. Jeong, *Food Chem. Toxicol.* **2014**, *71*, 51 and references cited therein. doi:10.1016/J.FCT.2014.06.001
For antiviral activities, see: (d) S. Loya, A. Hizi, *J. Biol. Chem.* **1993**, *268*, 9323.
(e) S. Loya, R. Tal, Y. Kashman, A. Hizi, *Antimicrob. Agents Chemother.* **1990**, *34*, 2009. doi:10.1128/AAC.34.10.2009

[4] (a) Ilimaquinone (**4**) was first isolated from *Hippiospongia metachromia* (see ref. [9] for taxonomy details). See: R. T. Luibrand, T. R. Erdman, J. J. Vollmer, P. J. Scheuer, J. Finer, J. Clardy, *Tetrahedron* **1979**, *35*, 609. doi:10.1016/0040-4020(79)87004-0
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[5] 5-*epi*-Ilimaquinone (**5**) was first isolated from a *Fenestraspongia* sp. specimen (which was 'in an unusually poor condition for identification'); see also ref. [4b] for stereochemical revision: B. Carté, C. B. Rose, D. J. Faulkner, *J. Org. Chem.* **1985**, *50*, 2785. doi:10.1021/JO00215A039

[6] 5,8-*epi*-Ilimaquinone (**6**) was recently isolated from *D. elegans* from the coast of Palau, see: L. Du, Y.-D. Zhou, D. G. Nagle, *J. Nat. Prod.* **2013**, *76*, 1175. doi:10.1021/NP400320R

[7] (a) Isospongiaquinone (**7**) was isolated from a sponge 'tentatively classified as *Stelospongia conulata*'; no stereochemistry was provided at that time, see: R. Kazlauskas, P. T. Murphy, R. G. Warren, R. J. Wells, J. F. Blount, *Aust. J. Chem.* **1978**, *31*, 2685. doi:10.1071/CH9782685
(b) The absolute stereochemistry of **7** was later determined by chemical correlations, see: R. J. Capon, *J. Nat. Prod.* **1990**, *53*, 753. doi:10.1021/NP50069A042

[8] (a) P. A. Takizawa, J. K. Yucel, B. Veit, D. J. Faulkner, T. Deerinck, G. Soto, M. Ellisman, V. Malhotra, *Cell* **1993**, *73*, 1079. doi:10.1016/0092-8674(93)90638-7
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[9] *Dactylospongia metachromia* de Laubenfels 1954 (Thorectidae), also formerly named *Hippospongia metachromia* de Laubenfels 1954 (Spongidae), see: R. van Soest, in *World Porifera Database* (Eds R. W. M. Van Soest, N. Boury-Esnault, J. N. A Hooper, K. Rützler, N. J. de Voogd, B. Alvarez de Glasby, E. Hajdu, A. B. Pisera, R. Manconi, C. Schoenberg, D. Janussen, K. R. Tabachnick, M. Klautau, B. Picton, M. Kelly, J. Vacelet, M. Dohrmann, M.-C. Díaz, P. Cárdenas) **2015**. Available at http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=165313 and http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=397152 (accessed 25 June 2015).

[10] *Dactylospongia elegans* Thiele 1899 (Thorectidae), also formerly named *Luffarella elegans* Thiele 1899 (Thorectidae), see: R. van Soest, in *World Porifera Database* (Eds R. W. M. Van Soest, N. Boury-Esnault, J. N. A. Hooper, K. Rützler, N. J. de Voogd, B. Alvarez de Glasby, E. Hajdu, A. B. Pisera, R. Manconi, C. Schoenberg, D. Janussen, K. R. Tabachnick, M. Klautau, B. Picton, M. Kelly, J. Vacelet, M. Dohrmann, M.-C. Díaz, P. Cárdenas) **2015**. Available at http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=165312 (accessed 25 June 2015).

[11] Cyanobacterial symbionts were shown to produce chlorinated metabolites, whereas sesquiterpenoids were found only in the sponge cells, see: M. D. Unson, D. J. Faulkner, *Experientia* **1993**, *49*, 349. doi:10.1007/BF01923420

[12] (a) Avarol (dihydro-**1**) and congeners were found within sponge choanocytes, see: M.-J. Uriz, X. Turon, J. Galera, J. M. Tur, *Cell Tissue Res.* **1996**, *285*, 519. doi:10.1007/S004410050668
(b) Avarol was produced from cultures of primmorphs from *Dysidea avara*, see: W. E. G. Müller, M. Böhm, R. Batel, S. De Rosa, G. Tommonaro, I. M. Müller, H. C. Schröder, *J. Nat. Prod.* **2000**, *63*, 1077. doi:10.1021/NP000003P
(c) Very interestingly, the variation of concentrations of avarol depending on time period and biotope-dependent factors was evaluated, see: S. De Caralt, D. Bry, N. Bontemps, X. Turon, M.-J. Uriz, B. Banaigs, *Mar. Drugs* **2013**, *11*, 489. doi:10.3390/MD11020489

[13] The gathering and cross-checking of data from literature dealing with ilimaquinones shows a variation of **4**/**5** ratio between publications but strangely this statement is never discussed as such based on multi-sample studies. Reports of co-isolation of **4** and **5** include *D. elegans* from Okinawa, Japan and **4**/**5** (96/4) ratio based on amounts presented in the experimental section after purification and not a ratio based on crude extract analysis, see: (a) H. Mitome, T. Nagasawa, H. Miyaoka, Y. Yamada, R. W. M. van Soest, *J. Nat. Prod.* **2001**, *64*, 1506. doi:10.1021/NP010299E
(b) *Dactylospongia elegans* from West Flores, Indonesia: **4**/**5** (3/5 ratio based on amounts presented in experimental section and not a ratio based on crude extract analysis), see: S. Aoki, D. Kong, K. Matsui, R. Rachmat, M. Kobayashi, *Chem. Pharm. Bull.* **2004**, *52*, 935. doi:10.1248/CPB.52.935
(c) *Dactylospongia elegans* from Truant Island, Australia: **4**/**5** (1/1 mixture, not separated), see: S. P. B. Ovenden, J. L. Nielson, C. H. Liptrot, R. H. Willis, D. M. Tapiolas, A. D. Wright, C. A. Motti, *J. Nat. Prod.* **2011**, *74*, 65. doi:10.1021/NP100669P
(d) *Dactylospongia elegans* from Fiji: only the presence of **5** is reported, see: J. Rodríguez, E. Quiñoa, R. Riguera, B. M. Peters, L. M. Abrell, P. Crews, *Tetrahedron* **1992**, *48*, 6667. doi:10.1016/S0040-4020(01)80012-0
(e) *Fenestraspongia* sp., Thorectidae from Urukthapel Island, Palau: **4**/**5** (6/4 mixture), see ref. [5].
(f) *Polyfibrospongia australis*, Thorectidae (now *Fasciospongia turgida*, Thorectidae) from Taiwan: mixture of **4** and **5** (no further precision). See: Y.-C. Shen, P.-W. Hsieh, *J. Nat. Prod.* **1997**, *60*, 93. doi:10.1021/NP9605302

[14] Having the same decalin ring system but with, apparently, opposite absolute configuration, asmarines **A**–**F** (isolated from a specimen of *Raspailia* sp.) bear diazepino-purines **a** in place of the quinone. They also exists as pairs of epimers at C-5 (asmarines **A**-**B**, **C**-**D**, **E**-**F** respectively), see: T. Yosief, A. Rudi, Y. Kashman, J. Nat. Prod. **2000**, *63*, 299. In the latter paper, the term '*epi*' is misleading and refers to a C-5 configuration (*S*), (*R*), (*S*) for asmarines **B**, **D**, **F**, respectively. No indication of the different ratios is given.

[15] The case of 5,8-di-*epi*-ilimaquinone (**6**) described recently by Nagle and coworkers is striking in terms of plausible biosynthetic pathway but is not discussed by the authors (see ref. [6]). It would apparently necessitate an unusual precursor i.e. *Z*,*E*-(**8**).

[16] (a) The lack of selectivity of terpene cyclases in terms of end products is now well documented. See for example: M. Köksal, Y. Jin, R. M. Coates, R. Croteau, D. W. Christianson, *Nature* **2011**, *469*, 116. doi:10.1038/NATURE09628
(b) See also, among others: V. Gonzalez, S. Touchet, D. J. Grundy, J. A. Faraldos, R. K. Allemann, *J. Am. Chem. Soc.* **2014**, *136*, 14505. doi:10.1021/JA5066366
[17] K. W. L. Yong, A. Jankam, J. N. A. Hooper, A. Suksamrarn, M. J. Garson, *Tetrahedron* **2008**, *64*, 6341. doi:10.1016/J.TET.2008.04.091
[18] (a) Neomamanuthaquinone **15** was isolated from a *Dactylospongia* sp. as a natural substance (see ref. [17]) but was described before as an semi-synthetic compound. See: (a) ref. [5],
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