

Article

## Nepheliosyne B, a New Polyacetylenic Acid from the New Caledonian Marine Sponge *Niphates* sp.

Nathalie Legrave<sup>1</sup>, Souhir Hamrouni-Buonomo<sup>1,2</sup>, Maeva Dufies<sup>3</sup>, Vincent Guérineau<sup>4</sup>, Jean Vacelet<sup>5</sup>, Patrick Auberger<sup>3</sup>, Philippe Amade<sup>1</sup> and Mohamed Mehiri<sup>1,\*</sup>

<sup>1</sup> Chemistry Institute of Nice (ICN), UMR 7272 CNRS, Bioactive Molecules, University of Nice-Sophia Antipolis, ParcValrose, F-06108 Nice Cedex 02, France; E-Mails: nathalie.legrave@unice.fr (N.L.); souhirhamrouni@yahoo.fr (S.H.-B.); philippe.amade@unice.fr (P.A.)

<sup>2</sup> UR Ecosystems and Aquatic Ressources (UR03AGRO1), Agronomic National Institute of Tunisia (INAT), Carthage University, 43 Avenue Charles Nicolle, Tunis 1082, Tunisia

<sup>3</sup> Mediterranean Centre of Molecular Medicine (C3M), INSERM UMR 1065, Team 2: Cell Death Differentiation and Cancer, Batiment ARCHIMED, 151 Route de Saint-Antoine de Ginestière, BP2 3194, 06204 Nice Cedex 3, France; E-Mails: maeva.dufies@gmail.com (M.D.); patrick.auberger@unice.fr (P.A.)

<sup>4</sup> Gif Research Centre, Chemistry Institute of Natural Substances, CNRS Avenue de la Terrasse, 91198 Gif-Sur-Yvette Cedex, France; E-Mail: vincent.guerineau@cnrs.fr

<sup>5</sup> Aix-Marseille University, CNRS UMR 7263, Mediterranean Institute of Biodiversity and Marine and Continental Ecology (IMBE), Station Marine d'Endoume, Rue de la Batterie des Lions, Marseille 13007, France; E-Mail: jean.vacelet@imbe.fr

\* Author to whom correspondence should be addressed; E-Mail: mehiri@unice.fr; Tel.: +33-492-076-154; Fax: +33-492-076-151.

Received: 3 May 2013; in revised form: 4 June 2013 / Accepted: 9 June 2013 /

Published: 27 June 2013

---

**Abstract:** A new C<sub>47</sub> polyoxygenated acetylenic acid, nepheliosyne **B** (**2**), along with the previously described nepheliosyne **A** (**1**), have been isolated from the New Caledonian marine sponge *Niphates* sp. Their structures have been elucidated on the basis of extensive spectroscopic analyses. These metabolites exhibited a moderate cytotoxicity against K562, U266, SKM1, and Kasumi cancer cell lines.

**Keywords:** marine natural products; highly oxygenated polyacetylenes; marine sponges; Porifera; Haplosclerida

## 1. Introduction

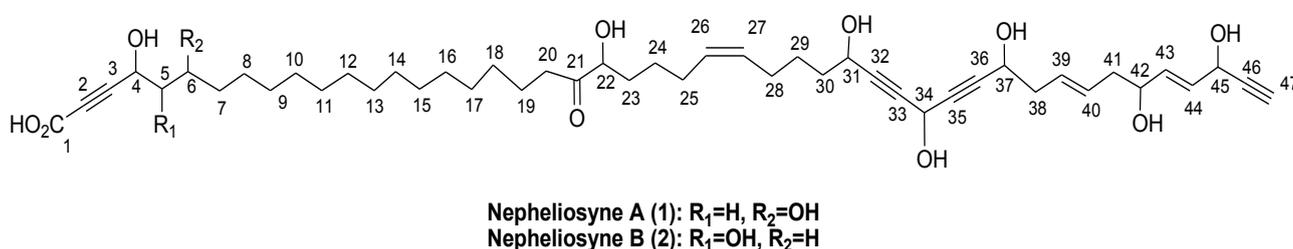
Several natural acetylenic metabolites, often featuring a polyketide or fatty acid origin, have been isolated from plants, fungi, marine algae, sponges, and tunicates [1]. The main source of oxygenated polyacetylenes is the phylum Porifera [2], especially the genera *Xestospongia* [3,4], *Petrosia* [5–9], and *Haliclona* [10–12]. The structure of these metabolites differs significantly from each other in terms of chain-length, functional groups as well as their locations in the carbon framework. Nepheliosyne **A** [3], petrosolic acid [6], osirisynes **A–F** [10], haliclonyne [11], and fulvynes **A–I** [12] are a few examples of linear acetylenes with a diacetylenic carbinol and an  $\alpha$ -yne carboxylic acid functionality as common structural features. Several of these sponge-derived polyacetylenes exhibit potent bioactivities including antiviral [6], antimicrobial [7,12], cytotoxic [8,9], and enzyme inhibitory [13] activities. Nepheliosyne **A** and petrosolic acid, isolated from *Xestospongia* sp. and *Petrosia* sp., respectively, are the only examples of oxygenated polyacetylene with diacetylenic carbinol,  $\alpha$ -hydroxyketone, and  $\alpha$ -yne carboxylic groups. Some of the reported data of nepheliosyne **A** remain hypothetical, specifically the size of the methylene link between the two groups  $\alpha$ -yne carboxylic and  $\alpha$ -hydroxyketone.

In the course of our search for bioactive marine natural products, we have investigated the New Caledonian marine sponge *Niphates* sp. (Haplosclerida, Niphatidae). In this paper, we report the isolation and structural elucidation of new polyhydroxylated acetylene, nepheliosyne **B** (**2**), along with nepheliosyne **A** (**1**), previously isolated from the marine sponge *Xestospongia* sp., whose structural elucidation is herein completed. We also report their cytotoxic properties against K562, U266, SKM1, and Kasumi cancer cell lines.

## 2. Results and Discussion

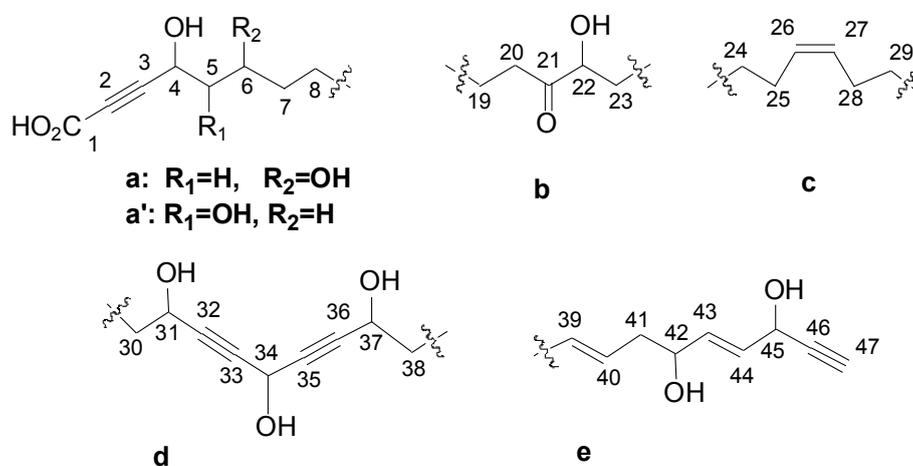
The CH<sub>2</sub>Cl<sub>2</sub>/MeOH (1:1, v/v) crude extract of *Niphates* sp. was fractionated by Flash Vacuum Liquid Chromatography, eluting with a gradient of decreasing polarity from H<sub>2</sub>O to MeOH. The subsequent MeOH fraction was purified by semi-preparative reverse-phase HPLC (Phenomenex Luna C18, 250 × 10 mm id, 5  $\mu$ m, gradient H<sub>2</sub>O/MeCN/Formic Acid 50:50:0.1 to 0:100:0.1) to afford pure nepheliosyne **A** (**1**) (8.7 mg) and nepheliosyne **B** (**2**) (6.7 mg) (Figure 1).

**Figure 1.** Structure of nepheliosynes **A** (**1**) and **B** (**2**).



Both compounds, obtained as amorphous solid, have the same molecular formula  $C_{47}H_{70}O_{11}$  deduced from the HR-MALDI-TOF data which showed for each a pseudomolecular ion adduct at  $m/z$  833.4803  $[M + Na]^+$ . The IR spectrum showed bands at 3450, 3300, 2250, 2100, and 1705  $cm^{-1}$  suggesting the presence of double bonds, triple bonds, and hydroxyl groups. A preliminary NMR spectral analysis showed similarities and strongly supported the presence of a polyhydroxylated acetylenic skeleton. The  $^1H$  and  $^{13}C$  NMR spectra of compound **1** (see Supporting Information and Table 1) revealed the following functional groups: (a) Seven non-protonated  $sp$  carbons ( $\delta_C$  87.3–78.0) and one terminal methine ( $\delta_C$  74.4;  $\delta_H$  2.90); (b) Six  $sp^2$  carbons ( $\delta_C$  135.7–127.5 and  $\delta_H$  5.91–5.38) suggesting three disubstituted carbon-carbon double bonds; (c) Eight oxymethine groups ( $\delta_C$  78.0 to 52.3,  $\delta_H$  5.17–3.79); and (d) A propargyl carboxylic group and a ketone identified from the carbon signals at  $\delta_C$  157.6 and 215.4, respectively. The relatively high field chemical shift of several of the eight oxymethine carbon resonances suggested their allylic and propargylic positions. The  $^1H$  and  $^{13}C$  NMR spectra of compound **2** (see Supporting Information and Table 2) allowed the identification of the same functional groups. These elements suggested partial structures **a** (for compound **1**), **a'** (for compound **2**), as well as the common parts **b–e** (Figure 2) to be identified as shown.

**Figure 2.** Partial structures (**a**, **a'**, **b–e**) of nepheliosynes **A (1)** and **B (2)**.



Fragment **a** in compound **1**, included two oxymethines at  $\delta_H$  4.64 (H-4,  $\delta_C$  59.3) and  $\delta_H$  3.84–3.79 (H-6,  $\delta_C$  68.4) linked together by a methylene at  $\delta_H$  1.86–1.71 (H<sub>2</sub>-5,  $\delta_C$  45.6). Proton at  $\delta_H$  3.84–3.79 (H-6,  $\delta_C$  68.4) was coupled to a methylene at  $\delta_H$  1.45 (H<sub>2</sub>-7,  $\delta_C$  38.7) in turn linked to another methylene at  $\delta_H$  1.30 (H<sub>2</sub>-8,  $\delta_C$  30.8–30.4). Heteronuclear Multiple Bond Correlation (HMBC) correlations were observed between, both H-4 ( $\delta_H$  4.64) and H-5 ( $\delta_H$  1.86–1.71) and C-3 ( $\delta_C$  87.3), and between H-4 and C-2 ( $\delta_C$  78.0) thus assigning the carbon resonances of the triple bond in fragment **a** (Table 1).

Fragment **a'** in compound **2**, was formed by two vicinal oxymethines at  $\delta_H$  4.26 (H-4,  $\delta_C$  67.1) and  $\delta_H$  3.58 (H-5,  $\delta_C$  74.9), itself coupled to methylenes at  $\delta_H$  1.69–1.44 (H<sub>2</sub>-6,  $\delta_C$  33.5) and at  $\delta_H$  1.29 (H<sub>2</sub>-7 and H<sub>2</sub>-8,  $\delta_C$  30.8–30.4). In a similar way than fragment **a** in compound **1**, HMBC correlations were observed between, both H-4 ( $\delta_H$  4.26) and H-5 ( $\delta_H$  3.58) and C-3 ( $\delta_C$  85.3), and between H-4 and C-2 ( $\delta_C$  80.1) thus assigning the carbon resonances of the triple bond in fragment **a'** (Table 2). The down-shifted proton value of H-4 in fragments **a** and **a'** suggested its connection to the  $\alpha$ -yne carboxylic acid moiety that had to be the first terminal part of the chain.

**Table 1.**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) data of nepheliosyne A (**1**).

Position	$\delta_{\text{C}}$ (ppm)/Multiplicity	$\delta_{\text{H}}$ (ppm)/ $J$ (Hz)/ Multiplicity	$^1\text{H}$ - $^1\text{H}$ COSY/TOCSY	$^1\text{H}$ - $^{13}\text{C}$ HMBC
1	157.7, qC			
2	78.0, qC			
3	87.3, qC			
4	59.3, CH	4.64, dd, 9.3, 3.5	5a, 6, 7	2, 3, 5, 6
5a	45.6, $\text{CH}_2$	1.74–1.71, m	4, 5b, 6, 7	3, 4, 7
5b	45.6, $\text{CH}_2$	1.86–1.81, m	4, 5a, 6, 7, 8	3, 4, 6, 7
6	68.4, CH	3.84–3.79, m	4, 5a, 5b, 7, 8	4, 5b
7	38.7, $\text{CH}_2$	1.45, m	4, 6, 5a, 5b, 7, 8	8
8–18	30.8–30.4, $\text{CH}_2$	1.30, m	5a, 5b, 7, 17, 19	
19	24.1, $\text{CH}_2$	1.59–1.55, m	18, 20, 24, 25	20, 24, 25
20	38.6, $\text{CH}_2$	2.61–2.49, m	18, 19, 25, 26	18, 19, 21
21	215.4, qC			
22	78.0, CH	4.05, dd, 8.1, 4.2	19, 23, 24	21, 23, 24
23a	34.6, $\text{CH}_2$	1.74–1.69, m	22, 24, 25, 26	20
23b	34.6, $\text{CH}_2$	1.61–1.49, m	22, 24, 25, 26	20
24	26.2, $\text{CH}_2$	1.40–1.35, m	20, 22, 23, 25, 26	19, 23, 25, 26, 27
25	28.0, $\text{CH}_2$	2.14–2.05, m	20, 23, 24, 26, 27	18, 19, 24, 26, 27
26	130.7, CH	5.38, m	20, 23, 24, 25, 28	25
27	130.9, CH	5.38, m	28, 29, 30, 31	28
28	27.8, $\text{CH}_2$	2.14–2.05, m	26, 27, 29, 30, 31	26, 27, 29, 30
29	26.7, $\text{CH}_2$	1.56–1.49, m	26, 27, 28, 30, 31	28, 30, 31
30	38.3, $\text{CH}_2$	1.70–1.64, m	26, 27, 28, 29, 31	28, 29, 31
31	62.6, CH	4.33, dd, 6.7, 1.4	26, 27, 28, 29, 30, 34	30, 32, 33
32	83.1, qC			
33	82.9, qC			
34	52.3, CH	5.17, t, 1.6	29, 30, 31, 37, 38	32, 33, 35, 36
35	85.6, qC			
36	85.9, qC			
37	62.6, CH	4.36, dd, 6.6, 1.6	34, 38, 39, 40	35, 38
38	36.8, $\text{CH}_2$	2.45, t, 6.1	31, 37, 39, 40	36, 37, 39, 40
39	129.2, CH	5.64–5.54, m	37, 38, 40, 41, 42	38, 42, 44
40	127.5, CH	5.64–5.54, m	37, 38, 39, 41, 42	41, 42, 44
41	36.3, $\text{CH}_2$	2.38–2.25, m	39, 40, 42	39, 40, 42, 43
42	72.2, CH	4.15, dd, 6.3	39, 40, 41, 44	39, 40, 41, 43, 44
43	135.7, CH	5.91, ddd, 15.4, 5.9, 1.3	41, 42, 44, 45	42, 44, 45
44	130.7, CH	5.78, ddd, 15.4, 5.7, 1.2	41, 42, 43, 45	42, 43, 45, 46
45	62.5, CH	4.83, d, 5.5	37, 42, 43, 44, 47	43, 44, 46, 47
46	84.4, qC			
47	74.4, CH	2.90, d, 2.0	45	44, 45

**Table 2.**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) and  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) data of nepheliosyne **B** (**2**).

Position	$\delta_{\text{C}}$ (ppm)/Multiplicity	$\delta_{\text{H}}$ (ppm)/ $J$ (Hz)/ Multiplicity	$^1\text{H}$ - $^1\text{H}$ COSY/TOCSY	$^1\text{H}$ - $^{13}\text{C}$ HMBC
1	157.7, qC			
2	80.1, qC			
3	85.3, qC			
4	67.1, CH	4.26, d, 5.02	5, 6, 7	1, 5
5	74.9, CH	3.58, m	4, 6, 7	4
6a	33.5, $\text{CH}_2$	1.69–1.64, m	4, 5, 7, 8	4, 5
6b	33.5, $\text{CH}_2$	1.52–1.44, m	4, 5, 7, 8	4, 5
7	30.8–30.4, $\text{CH}_2$	1.29, m	4, 5, 6, 8	8
8–18	30.8–30.4, $\text{CH}_2$	1.29, m	5a, 5b, 7, 17, 19	
19	24.1, $\text{CH}_2$	1.59–1.55, m	18, 20, 24, 25	20, 24, 25
20	38.6, $\text{CH}_2$	2.61–2.49, m	18, 19, 25, 26	18, 19, 21
21	215.0, qC			
22	78.0, CH	4.05, dd, 8.1, 4.2	19, 23, 24	21, 23, 24
23a	34.6, $\text{CH}_2$	1.57–1.50, m	22, 24, 25, 26	20
23b	34.6, $\text{CH}_2$	1.73–1.67, m	22, 24, 25, 26	20
24	25.9, $\text{CH}_2$	1.42–1.34, m	20, 22, 23, 25, 26	19, 23, 25, 26, 27
25	28.0, $\text{CH}_2$	2.14–2.05, m	20, 23, 24, 26, 27	18, 19, 24, 26, 27
26	130.7, CH	5.38, m	20, 23, 24, 25, 28	25
27	130.9, CH	5.38, m	28, 29, 30, 31	28
28	27.8, $\text{CH}_2$	2.14–2.05, m	26, 27, 29, 30, 31	26, 27, 29, 30
29	26.2, $\text{CH}_2$	1.58–1.49, m	26, 27, 28, 30, 31	28, 30, 31
30	38.6, $\text{CH}_2$	1.70–1.62, m	26, 27, 28, 29, 31	28, 29, 31
31	62.6, CH	4.33, dd, 6.7, 1.4	26, 27, 28, 29, 30, 34	30, 32, 33
32	83.1, qC			
33	82.9, qC			
34	52.4, CH	5.17, t, 1.6	29, 30, 31, 37, 38	32, 33, 35, 36
35	85.6, qC			
36	85.9, qC			
37	62.6, CH	4.36, dd, 6.6, 1.6	34, 38, 39, 40	35, 38
38	36.9, $\text{CH}_2$	2.45, t, 6.1	31, 37, 39, 40	36, 37, 39, 40
39	127.6, CH	5.64–5.54, m	37, 38, 40, 41, 42	38, 42, 44
40	129.2, CH	5.64–5.54, m	37, 38, 39, 41, 42	41, 42, 44
41	36.4, $\text{CH}_2$	2.38–2.25, m	39, 40, 42	39, 40, 42, 43
42	72.2, CH	4.15, dd, 6.3	39, 40, 41, 44	39, 40, 41, 43, 44
43	135.7, CH	5.91, ddd, 15.4, 5.9, 1.3	41, 42, 44, 45	42, 44, 45
44	130.7, CH	5.78, ddd, 15.4, 5.7, 1.2	41, 42, 43, 45	42, 43, 45, 46
45	62.6, CH	4.83, d, 5.5	37, 42, 43, 44, 47	43, 44, 46, 47
46	84.4, qC			
47	75.1, CH	2.90, d, 2.0	45	44, 45

Structural elucidations of the partial structures **b–e** hereafter are based on those of compound **1** as  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of these fragments are almost identical in both compounds.

Fragment **b** contained a ketone group flanked by one adjacent methylene at  $\delta_{\text{H}}$  2.61–2.49 (H<sub>2</sub>-20,  $\delta_{\text{C}}$  38.6) and an oxymethine at  $\delta_{\text{H}}$  4.05 (H<sub>2</sub>-22,  $\delta_{\text{C}}$  78.0), which are correlated to methylenes at  $\delta_{\text{H}}$  1.59–1.55 (H<sub>2</sub>-19,  $\delta_{\text{C}}$  24.1) and at  $\delta_{\text{H}}$  1.74–1.49 (H<sub>2</sub>-23,  $\delta_{\text{C}}$  34.6), respectively.

Fragment **c** was constituted by the olefinic protons at  $\delta_{\text{H}}$  5.38 (H-26,  $\delta_{\text{C}}$  130.7 and H-27,  $\delta_{\text{C}}$  130.9), which are correlated to methylenes at  $\delta_{\text{H}}$  2.14–2.05 (H<sub>2</sub>-25,  $\delta_{\text{C}}$  28.0 and H<sub>2</sub>-28,  $\delta_{\text{C}}$  27.8). HMBC correlations were observed between these latter and H<sub>2</sub>-24 ( $\delta_{\text{H}}$  1.40–1.35  $\delta_{\text{C}}$  26.2) and H<sub>2</sub>-29 ( $\delta_{\text{H}}$  1.56–1.49  $\delta_{\text{C}}$  26.4), respectively.

Fragment **d** consisted of the oxymethines at  $\delta_{\text{H}}$  4.36 (H-37,  $\delta_{\text{C}}$  62.6) and  $\delta_{\text{H}}$  4.33 (H-31,  $\delta_{\text{C}}$  62.6), which correlated to the methylenes at  $\delta_{\text{H}}$  2.45 (H-38,  $\delta_{\text{C}}$  36.8) and at  $\delta_{\text{H}}$  1.70–1.64 (H<sub>2</sub>-30,  $\delta_{\text{C}}$  38.3), respectively. These protons showed a long-range coupling to the oxymethine located between two triple bonds (H-34,  $\delta_{\text{H}}$  5.17,  $\delta_{\text{C}}$  52.3). HMBC correlations were observed between C-32 ( $\delta_{\text{C}}$  83.1) and both H-31 ( $\delta_{\text{H}}$  4.33) and H-34 ( $\delta_{\text{H}}$  5.17), and between C-36 ( $\delta_{\text{C}}$  85.9) and methines at  $\delta_{\text{H}}$  4.36 (H-37) and H-34 ( $\delta_{\text{H}}$  5.17), whereas the signal at  $\delta_{\text{C}}$  82.9 (C, C-33 and C-35) showed cross-peaks only with H-34 ( $\delta_{\text{H}}$  5.17) thus allowing the carbon assignment of the two triple bonds in the bis propargylic alcohol.

Fragment **e** was established to be formed sequentially by the acetylenic proton at  $\delta_{\text{H}}$  2.90 (H-47,  $\delta_{\text{C}}$  74.4), which constitutes the second terminus of the chain, the oxymethine at  $\delta_{\text{H}}$  4.83 (H-45,  $\delta_{\text{C}}$  62.5), the olefinic signals at  $\delta_{\text{H}}$  5.78 (H-44,  $\delta_{\text{C}}$  130.7) and  $\delta_{\text{H}}$  5.91 (H-43,  $\delta_{\text{C}}$  135.7), the oxymethine at  $\delta_{\text{H}}$  4.15 (H-42,  $\delta_{\text{C}}$  72.2), and the methylene at 2.38–2.25 (H<sub>2</sub>-41,  $\delta_{\text{C}}$  36.3). H<sub>2</sub>-41 is correlated to the olefinic protons at  $\delta_{\text{H}}$  5.64–5.54 (H-39,  $\delta_{\text{C}}$  129.2 and H-40,  $\delta_{\text{C}}$  127.5). In a similar way, HMBC correlations were observed between H-42 ( $\delta_{\text{H}}$  4.15) and C-41 ( $\delta_{\text{C}}$  36.3), C-43 ( $\delta_{\text{C}}$  135.7), and C-44 ( $\delta_{\text{C}}$  130.7).

The connectivities between these partial structures **a–e** for **1** and **a'–e** for **2**, as well as the number of the linking methylene groups, were established on the basis of the <sup>1</sup>H-<sup>13</sup>C HMBC, <sup>1</sup>H-<sup>1</sup>H COSY/TOCSY correlations, and MS data. The correlation observed between H<sub>2</sub>-23 and H<sub>2</sub>-25 offered the connection between fragments **b** and **c**. In a similar way, the correlation between H<sub>2</sub>-28 and H<sub>2</sub>-30 provided the connection between the partial structures **c** and **d**. Finally, the correlation between H-37 and H-39 offered the connection between the partial structures **d** and **e**. The combinations **a + b + c + d + e** and **a' + b + c + d + e** represented 670 m.u. whereas the molecular structure weight was 810 m.u. The difference corresponding to 10 methylene groups determined the length of the complementary alkyl chain between **a** (or **a'**) and **b**. All the spectral data of **1** (1D and 2D NMR, MS, and optical properties) led to its identification as nepheliosyne **A**, in accordance with previous published data [3]. Thus, nepheliosyne **B** (**2**) is a new metabolite defined as the 5-hydroxy-6-dehydroxy derivative of nepheliosyne **A** (**1**).

The geometry of the double bond  $\Delta^{43,44}$  was easily assigned as *E* by analysis of the coupling constant of the olefinic protons ( $J_{43,44} = 15.5$  Hz). The geometries of the double bond  $\Delta^{26,27}$  and  $\Delta^{39,40}$  were assigned as *Z* and *E*, respectively, based on the <sup>13</sup>C chemical shifts of the allylic methylenes  $\delta_{\text{C}}$  28.0 (C-25) and 27.8 (C-28) for  $\Delta^{26,27}$  and  $\delta_{\text{C}}$  36.8 (C-38) and 36.3 (C-41) for  $\Delta^{39,40}$ .

The relative and absolute configurations of the stereogenic centers of nepheliosyne **A** (**1**) and **B** (**2**) remained unassigned. They were observed also to degrade rapidly under different reaction conditions [14–16]. Like fulvynes [12], any attempts to obtain suitable derivatives for a stereochemical analysis were unsuccessful.

To date, several polyhydroxylated acetylenic metabolites of marine sponges with a diacetylenic carbinol and a  $\alpha$ -yne carboxylic group have been reported: Nepheliosyne **A** from *Xestospongia* sp. [3], petrosolic acid from *Petrosia* sp. [6], osirisynes [10] and haliclonyne [11] from *Haliclona* sp., and fulvynes **A–I** from *Haliclona fulva* [12]. Nepheliosyne **B** (**2**) is, with nepheliosyne **A** and petrosolic acid, the third example of linear acetylene with diacetylenic carbinol,  $\alpha$ -hydroxyketone, and  $\alpha$ -yne carboxylic groups. To the best of our knowledge this is the first report on the isolation and structure identification of oxygenated polyacetylenic metabolites from *Niphates* sp. All these data suggest that from a chemotaxonomic point of view polyhydroxylated acetylenic metabolites could constitute potential markers of Haplosclerida species.

The cytotoxicity of the nepheliosynes **A** (**1**) and **B** (**2**) was evaluated against K562, U266, SKM1, and Kasumi cancer cell lines which are widely used for cytotoxicity assays, and the  $IC_{50}$  values in  $\mu$ M (XTT assay) are indicated in Table 3. Compounds **1** and **2** were equally efficient with  $IC_{50}$  values around 150–200  $\mu$ M. Interestingly, their significant specificity against tumor cells were highlighted thanks to additional assays showing that, compared to the K562 cells, the peripheral blood mononuclear cells (PBMC) viability is not affected by compounds **1** and **2** (see Supporting Information).

**Table 3.**  $IC_{50}$  values for compounds **1** and **2** for loss of cell metabolism (XTT assay) and cell number. Cells ( $10 \times 10^4$ /mL) were incubated for 48 h at 37 °C with either increasing concentrations of compounds **1** and **2** in the 2.5–250  $\mu$ M range. Cell metabolism was measured using the XTT kit assay as indicated in the Experimental section.  $IC_{50}$  values are representative of three experiments made in quadruplicates.

Compounds	SKM1 ( $IC_{50}$ )	U266 ( $IC_{50}$ )	Kasumi ( $IC_{50}$ )	K562 ( $IC_{50}$ )
1	250	170	200	200
2	>250	200	150	150

### 3. Experimental Section

#### 3.1. General

All organic solvents used for material extraction were of analytical grade and purchased from Merck (Darmstadt, Germany). Acetonitrile used for HPLC was of HPLC-grade and purchased from Fisher Scientific, USA. Formic acid of HPLC grade was purchased from Acros, USA. 2,5-Dihydroxybenzoic acid (DHB), used as the matrix for MALDI-TOF experiments, was of the highest grade available and used without further purification was purchased from Sigma Aldrich Co, France. The Chromabond C18 preparative column used for flash chromatography was obtained from Merck, USA. UV measurements were performed on a Varian Cary 300 Scan UV-visible spectrometer. IR spectra were obtained with a Perkin-Elmer Spectrum 100 series FT-IR spectrometer equipped with an universal attenuated total reflectance sampling accessory (ATR). Optical rotations were measured on a Jasco P-1010 polarimeter. Flash chromatography was performed on an Armen Instrument Spot Liquid Chromatography system, the detection wavelength was set at 254 nm. HPLC purifications were carried out on a Waters 600 system equipped with a Waters 717 plus autosampler, a Waters 996 photodiode array detector, and a Sedex 55 evaporative light-scattering detector (SEDERE, Alfortville,

France). Detection wavelengths were set at 214, 254 and 280 nm.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with 500 MHz Bruker Avance NMR spectrometers. Chemical shifts ( $\delta$ ) are recorded in ppm with  $\text{CD}_3\text{OD}$  ( $\delta_{\text{H}}$  3.31 ppm and  $\delta_{\text{C}}$  49.0 ppm) as internal standard with multiplicity (s singlet, d doublet, t triplet, m multiplet). High resolution mass spectra (HRMS) were conducted on a Voyager DE-STR MALDI-TOF mass spectrometer (ABSciex, Les Ulis, France), equipped with a 337 nm pulsed nitrogen laser (20 Hz) and a Acquiris<sup>®</sup> 2 GHz digitizer board, was used for all experiments. Mass spectra were obtained in reflectron positive ion mode with the following settings: Accelerating voltage 20 kV, grid voltage 62% of accelerating voltage, extraction delay time of 100 ns. The laser intensity was set just above the ion generation threshold to obtain peaks with the highest possible signal-to-noise (S/N) ratio without significant peak broadening. All data were processed using the Data Explorer software package (ABSciex).

### 3.2. Sponge Material

We collected the sponge *Niphates* sp. (Schmidt, 1862) (Demospongiae, Haplosclerida, Niphatidae) in November 2008 using scuba at a depth of 22 m in the south-west lagoon of New Caledonia (Philippe Amade, Figure 3). The sponge was identified by J. Vacelet and a voucher specimen (MHNM 1646) was deposited at the Natural History Museum of Marseille (France) [17].

**Figure 3.** *Niphates* sp. (photo: Philippe Amade).



### 3.3. Extraction and Isolation

A portion of *Niphates* sp. was freeze-dried and ground to obtain a dry powder (28 g), which was exhaustively extracted with a mixture of  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  (1:1, v/v) to yield 463 mg of the crude extract after concentration under reduced pressure. The crude extract was fractionated by RP-C18 flash chromatography (elution with a decreasing polarity gradient of  $\text{H}_2\text{O}/\text{MeOH}$  from 1:0 to 0:1, then  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  from 1:0 to 0:1) (flow:  $10 \text{ mL}\cdot\text{min}^{-1}$ ). The MeOH fraction (100 mg) was then subjected to semi-preparative HPLC-DAD (Phenomenex Luna C18,  $250 \times 10 \text{ mm id}$ ,  $5 \mu\text{m}$ ) with a gradient of  $\text{H}_2\text{O}/\text{MeCN}/\text{Formic acid}$  50:50:0.1 to 0:100:0.1 (flow:  $3.0 \text{ mL}\cdot\text{min}^{-1}$ , injection volume:  $100 \mu\text{L}$ ) to afford the pure compounds **1** and **2**. Both were identified by a combination of spectroscopic methods (1D and 2D NMR, MS) and comparison with the literature data [3–12].

### 3.4. Characterization Data

Nepheliosyne **A**: amorphous solid;  $[\alpha]_D = +7.0$  ( $c$  0.01, MeOH); IR (solid) 3450, 3300, 2250, 2100, 1705  $\text{cm}^{-1}$ ; UV  $\lambda_{\text{max}}$  (MeOH) 205 nm ( $\epsilon$  2500); HR-MALDITOF-MS  $m/z$  833.4803  $[\text{M} + \text{Na}]^+$  (Calcd. for  $\text{C}_{47}\text{H}_{70}\text{O}_{11}\text{Na}$  833.4726,  $\Delta = -1.05$  ppm); For  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) data see Table 1.

Nepheliosyne **B**: amorphous solid;  $[\alpha]_D = +9.9$  ( $c$  0.01, MeOH); IR (solid) 3450, 3300, 2250, 2100, 1705  $\text{cm}^{-1}$ ; UV  $\lambda_{\text{max}}$  (MeOH) 205 nm ( $\epsilon$  2500); HR-MALDITOF-MS  $m/z$  833.4803  $[\text{M} + \text{Na}]^+$  (Calcd. for  $\text{C}_{47}\text{H}_{70}\text{O}_{11}\text{Na}$  833.4726,  $\Delta = -1.05$  ppm); For  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) data see Table 2.

### 3.5. Biological Activity

Cell Lines. The human cancer cell lines K562 (chronic myelogenous leukemia), U266 (myeloma), SKM1 (myelodysplastic syndrom), and Kasumi (acute myeloid leukemia) were provided by American Type Culture Collection (ATCC) and were grown at 37 °C under 10%  $\text{CO}_2$  in RPMI 1640 medium (Gibco BRL, Paisley, UK) supplemented with 10% Fetal Calf Serum (FCS) (Gibco BRL, Paisley, UK) completed with 50 units/mL penicillin, 50 mg/mL streptomycin and 1 mM sodium pyruvate.

Measurement of Cell Metabolism (XTT). Cells ( $10 \times 10^4/\text{mL}$ ) were incubated with **1** or **2** for the times indicated. 50  $\mu\text{L}$  of XTT kit (sodium 39-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro)benzene sulfonic acid hydrate) was added to each well, which contain 100  $\mu\text{L}$  of medium. Absorbance of the formazan dye produced by metabolically active cells was measured at 490 nm as described earlier [18]. Each assay was performed in quadruplicate.

## 4. Conclusions

Nepheliosyne **B** (**2**), a new C47 highly oxygenated polyacetylene, along with nepheliosyne **A** (**1**), have been isolated from the New Caledonian marine sponge *Niphates* sp. Their structures have been determined on the basis of extensive spectroscopic analyses which led us to complete the structure determination of the previously reported nepheliosyne **A** (**1**). With a significant specificity, these metabolites exhibited a moderate cytotoxicity against K562, U266, SKM1, and Kasumi cancer cell lines.

## Acknowledgments

We are grateful to French “Ministère de l’Enseignement Supérieur et de la Recherche” and Campus France “AVERROES” for financial support to Nathalie Legrave and Souhir Hamrouni, respectively, and to Pharmamar for financial help for the sponge collection. Thanks are also to the “Direction de l’Environnement de la Province Sud” in New Caledonia, for assistance in collecting the marine samples. Finally, we are grateful to Clemence Ginet for the additional assays on PBMC cells.

## Conflict of Interest

The authors declare no conflict of interest.

## References and Notes

1. Minto, R.E.; Blacklock, B. Biosynthesis and function of polyacetylenes and allied natural products. *J. Prog. Lipid Res.* **2008**, *47*, 233–306.
2. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2011**, *28*, 196–268.
3. Kobayashi, J.; Naitoh, K.; Ishida, K.; Shigemori, H.; Ishibashi, M. Nepheliosyne A, new C<sub>47</sub> acetylenic acid from the Okinawan marine sponge *Xestospongia* sp. *J. Nat. Prod.* **1994**, *57*, 1300–1303.
4. Brantley, S.E.; Molinski, T.F.; Preston, C.M.; DeLong, E.F. Brominated acetylenic fatty acids from *Xestospongia* sp., a marine sponge-bacteria association. *Tetrahedron* **1995**, *51*, 7667–7672.
5. Jung, S.-K.; Young, J.-L.; Kwang, S.-I.; Jee, H.-J.; Chung, J.-S.; Chong, O.-L.; Jongki, H.; Hongkum, L. Cytotoxic polyacetylenes from the marine sponge *Petrosia* sp. *J. Nat. Prod.* **1999**, *62*, 554–559.
6. Isaacs, S.; Kashman, Y.; Loya, S.; Hizi, A.; Loya, Y. Petrosynol and petrosolic acid, two novel natural inhibitors of the reverse transcriptase of human immunodeficiency virus from *Petrosia* sp. *Tetrahedron* **1993**, *49*, 10435–10438.
7. Li, H.-Y.; Matsunaga, S.; Fusetani, N. Corticatic acids A–C, antifungal acetylenic acids from the marine sponge, *Petrosia corticata*. *J. Nat. Prod.* **1994**, *57*, 1464–1467.
8. Ueoka, R.; Ise, Y.; Matsunaga, S. Cytotoxic polyacetylenes related to petroformyne-1 from the marine sponge *Petrosia* sp. *Tetrahedron* **2009**, *65*, 5204–5208.
9. Okamoto, C.; Nakao, Y.; Fujita, T.; Iwashita, T.; van Soest, R.W.M.; Fusetani, N.; Matsunaga, S. Cytotoxic C<sub>47</sub>-polyacetylene carboxylic acids from a marine sponge *Petrosia* sp. *J. Nat. Prod.* **2007**, *70*, 1816–1819.
10. Shin, J.; Seo, Y.; Cho, K.W.; Rho, J.-R.; Paul, V.J. Osirisynes A–F, highly oxygenated polyacetylenes from the sponge *Haliclona osiris*. *Tetrahedron* **1998**, *54*, 8711–8720.
11. Chill, L.; Miroz, A.; Kashmann, Y. Haliclonyne, a new highly oxygenated polyacetylene from the marine sponge *Haliclona* species. *J. Nat. Prod.* **2000**, *63*, 523–526.
12. Nuzzo, G.; Ciavatta, M.L.; Villani, G.; Manzo, E.; Zanfardino, A.; Varcamonti, M.; Gavagnin, M. Fulvynes, antimicrobial polyoxygenated acetylenes from the Mediterranean sponge *Haliclona fulva*. *Tetrahedron* **2012**, *68*, 754–760.
13. Nakao, Y.; Uehara, T.; Matsunaga, S.; Fusetani, N.; van Soest, R.W.M. Callyspongynic acid, a polyacetylenic acid which inhibits  $\alpha$ -glucosidase, from the marine sponge *Callyspongia truncata*. *J. Nat. Prod.* **2002**, *65*, 922–924.
14. Sullivan, G.R.; Dale, J.A.; Mosher, H.S. Correlation of configuration and fluorine-19 chemical shifts of alpha-methoxy-alpha-trifluoromethylphenyl acetate derivatives. *J. Org. Chem.* **1973**, *38*, 2143–2147.
15. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-field FT NMR application of Mosher's method. The absolute configurations of marine terpenoids. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
16. Any attempt to obtain the absolute configuration of C-22 by circular dichroism was also unsuccessful.

17. Natural History Museum of Marseille (France). Available online: <http://www.museum-marseille.org/> (accessed on 17 June 2013).
18. Puissant, A.; Grosso, S.; Jacquel, A.; Belhacene, N.; Colosetti, P.; Cassuto, J.P.; Auberger, P. Imatinib mesylate-resistant human chronic myelogenous leukemia cell lines exhibit high sensitivity to the phytoalexin resveratrol. *FASEB J.* **2008**, *22*, 1894–1904.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).