Sporochartines A–E, A New Family of Natural Products from the Marine Fungus Hypoxylon monticulosum Isolated from a Sphaerocladina Sponge

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Four new sporochartines B–E were isolated from the marine fungus Hypoxylon monticulosum CLL-205, isolated from a sponge belonging to the Sphaerocladina order and collected in Tahiti coast. Sporochartine A (1), the first representative of this family was previously isolated from the same fungus. The structures of sporochartines B–E were elucidated using 1D and 2D NMR, HRMS and IR data. Their configurations were established according to ROE correlations and comparison with the absolute configuration of sporochartine A (1) previously obtained from X-ray analysis. Sporochartines A–D (2–4) may be derived from endo Diels-Alderase type catalysis and sporochartine E (5) from an exo Diels-Alderase catalysis. The spatial conformation of sporochartines drastically influences the results of the cytotoxic bioassay against HCT-116, PC-3, and MCF-7 human cancer cell lines.

Keywords: Hypoxylon, Sphaerocladina, sporothriolide, sporochartines, cytotoxic compounds

INTRODUCTION

The fungal Xylariaceae family includes more than 16 genera and 130 species (Sánchez-Ballesteros et al., 2000) and has been extensively investigated for the chemo diversity and biological activity of their metabolites (Stadler et al., 2006, 2008). Among the 16 genera reported, Hypoxylon with 14 species is largely distributed in various marine and terrestrial habitats, and producing a large variety of bioactive compounds among which cohaerins (Quang et al., 2005a; Surup et al., 2013), daladinins and daladinones (Quang et al., 2004; Gu et al., 2007), cytochalasin (Espada et al., 1997), fragiformin (Stadler et al., 2006), mitorubrinols (Quang et al., 2005b), hypoxylonols (Fukai et al., 2012), hypoxylans (Kuhnert et al., 2015a), hypoxymelhotins (Kuhnert et al., 2014), rickenyls (Kuhnert et al., 2015b), rutilins (Quang et al., 2005b), carneic acids (Quang et al., 2006), hymatoxins (Bodo et al., 1987; Borgschulte et al., 1991), malettinins (Angawi et al., 2005), hypoxysordarin (Daferner et al., 1999), lenormandins (Kuhnert et al., 2015c), nodulisporic acids (Bills et al., 2012), schweinitzin A (Linh et al., 2014), truncatones (Sudarman et al., 2016), macrocyclic polyesters 15G256 family (Schlingmann et al., 2002), and sporothriolide (Krohn et al., 1994; Surup et al., 2014; Cao et al., 2016).

Sporothriolide belongs to the furofurandione family of natural compounds first published in 1994 (Krohn et al., 1994). This compound exhibits antifungal activity and benefits from substantial
The sporothriolide is related to Sporothrix sp. Hektoen and Perkins (strain 700), from which this compound was first isolated. Sporothrix genus belongs to a different ascomycete family, ophiostomataceae. The first report on sporothriolide in 1994 detailed both the structure and bioactivity of this product (Krohn et al., 1994). It shows that Sporothrix produces sporothriolide, dihydrosporothriolide, as well as various sporothriolide analogs with different side-chain length (canadensolide, discosiolide, avenaciolide, ethiosolide). The authors reported also the antifungal/herbicidal activities of these compounds (Krohn et al., 1994) (Figure 1).

Twenty years later, sporothriolide and dihydrosporothriolide were isolated from Hypoxylon monticulosum together with three monocyclic acid precursors: sporothric acid, isosporothric acid and dihydroisosporothric acid (Figure 1) (Surup et al., 2014). More recently, sporothriolide was isolated from Nodulisporium sp., an anamorph of Hypoxylon, and the herbicidal activity was confirmed (Cao et al., 2016).

In our previous contribution, we added to the scarce sporothriolide family two new compounds, deoxysporothric acid and a new complex architecture sporochartine A, combining sporothriolide and trienylnuranol A moieties. The trienylnuranol A was recently isolated from a different Hypoxylon submoniticulosum (Burgess et al., 2017).

In the present work we report four new sporochartines B to E. Their structures were elucidated using 1D and 2D NMR, HRMS, IR and comparison with sporochartine A data, for which the absolute configuration was previously established by X-Ray analysis. A Diels-Alderase type reaction is probably involved in the biosynthesis of the five isolated sporochartines, as discussed below.

The human cancer cell-lines cytotoxicity bioassay shows that the conformation of sporochartines has an impact on the biological activity.

**RESULTS AND DISCUSSION**

According to our previous work, sporochartine A (1) was obtained after 5 days cultivation of H. monticulosum CLL-205 in PDB broth (Leman-Loubière et al., 2017). By extending the cultivation of the same microorganism by a further 4 days, the...
ethyl acetate extract gives the HPLC chromatogram presented in Figure 2.

Sporochartines B–D were isolated as white powders by flash chromatography followed by semi-preparative HPLC. They had similar \([M+H]^+\) HRESIMS molecular weights, molecular formula \(C_{24}H_{34}O_6\) and IR spectra compared with sporochartine A (1) (Leman-Loubière et al., 2017). The \(^1\)H and \(^13\)C NMR spectra of sporochartines B–D were similar to those of compound 1 (Tables 1, 2). Optical rotations \([\alpha]_D^{25}\), IR bands and HRESIMS are reported in Table 2.

COSY and HMBC spectra, confirmed that sporochartines A–D had the same connectivities supporting similar planar scaffold (Figure 3). In addition, the common coupling constant of 15.4–15.6 Hz between H-18 and H-19 confirmed that the double bond C-18/C-19 is in \(E\) configuration.

Based on the previously reported absolute configuration of sporochartine A (1) and ROE correlations, we deduced the absolute configuration of sporochartines B–D (2–4) (Figure 4).

The common ROE correlations between H-2 and H-5 and between H-5 and H-6 requiring a \(cis\) orientation of these three protons was found in the sporochartine A–D. Therefore, the stereochemistry of the sporothriolide moiety was identical. Moreover, based on ROE correlations between H-20 and H-21 and between H-21 and H-23, the stereochemistry of the tetrahydrofuran moiety is also a common feature in sporochartines A–D.

For sporochartine B (2) (Figure 4), we did not observe ROE correlations between H-17 and H-2 and between H-17 and H-14b as in sporochartine A (1), while a new correlation is observed between H-17 and H-13. This data suggests that the carbon C-17 have opposite stereochemistry compared to 1 supporting a 3S,17R configuration of 2 (instead of 3S,17S in 1).

For sporochartine C (3) (Figure 4), the H-17/H-2 and H-17/H-14b correlations observed in sporochartine A (1) are absent. In addition, we observed a correlation between H-17 and H-13 and H-2 and H-14a in compound 3. Based on this data, we suggest that compound 3 has a 3R,17S configuration.

Sporochartine D (4) (Figure 4) conserved the correlations between H-17 and H-2 and between H-17 and H-14b reported for sporochartine A (1). Furthermore, the correlation between H-17 and H-13 is absent in both 4 and 1. In 4 we have an additional correlation between H-13 and H-2, absent in 1. These observations support the conclusion that 4 is the 3R,17R isomer of 1.

A new compound referred as sporochartine E (5) was also isolated as a white powder. Compound 5 has the same molecular formula \(C_{24}H_{34}O_6\) as compound 1, deduced from HRESIMS \(m/z\) [M+H]\(^+\) 419.2433. Here again we have eight degrees of unsaturation accounting for two \(\gamma\)-lactones, two double bonds, one six-membered cycle moiety and one tetrahydrofuran moiety.

**FIGURE 2 |** HPLC analysis of the ethyl acetate extract of Hypoxylon monticulosum CLL-205 cultivated in PBD for 9 days. Percentages were deduced from peak integration based on Light Scattering Detection chromatogram (LSD).
### Table 1 $^1$H NMR data for sporochartines A–E (Data acquired in CDCl$_3$ at 500 MHz).

| Position | Sporochartine A | Sporochartine B | Sporochartine C | Sporochartine D | Sporochartine E |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|
|          | $\delta_H$ mult, ($J$ in Hz) | $\delta_H$ mult, ($J$ in Hz) | $\delta_H$ mult, ($J$ in Hz) | $\delta_H$ mult, ($J$ in Hz) | $\delta_H$ ($J$ in Hz) |
| 2        | 3.32, d (5.3)   | 3.30, d (5.8)   | 3.24, d (5.9)   | 3.30, d (5.2)   | 3.42, d (5.9)   |
| 5        | 5.10, dd (3.5, 5.3) | 5.13, dd (4.3, 5.9) | 5.01, dd (4.2, 5.8) | 5.09, dd (3.6, 5.2) | 4.97, dd (4.1, 5.8) |
| 6        | 4.45, m         | 4.39, m         | 4.40, m         | 4.45, m         | 4.44, m         |
| 7        | 1.78, m         | 1.76, m         | 1.76, m         | 1.82, m         | 1.81, m         |
| 8        | 1.91, m         | 1.85, m         | 1.85, m         | 1.91, m         | 1.89, m         |
| 9        | 1.45, m         | 1.45, m         | 1.45, m         | 1.47, m         | 1.44, m         |
| 10       | 1.37, m         | 1.34, m         | 1.34, m         | 1.37, m         | 1.36, m         |
| 11       | 1.30, m         | 1.29, m         | 1.29, m         | 1.30, m         | 1.29, m         |
| 12       | 1.30, m         | 1.29, m         | 1.29, m         | 1.30, m         | 1.28, m         |
| 13       | 1.96, m         | 1.72, d (9.5, 14.1) | 2.02, brd d (5.8, 14.0) | 1.96, m         | 1.72, dd (9.5, 14.1) |
|          | 2.04, m         | 2.13, m         | 2.04, m         | 2.12, m         | 2.26, dd (6.0, 14.6) |
| 14       | 2.27, m         | 2.25, m         | 2.24, brd (21.4) | 2.26, brd (19.1) | 3.25, m         |
| 15       | 5.94, brd (9.9) | 5.95, brd (10.9) | 5.95, brd (9.9) | 5.94, brd (9.9) | 6.16, d (10.2) |
| 16       | 5.63, m         | 5.54, brd (10.9) | 5.50, dq (2.0, 9.9) | 5.51, m         | 5.56, ddd (2.2, 4.7, 5.5) |
| 17       | 2.76, brt (5.8) | 3.23, br m      | 3.19, br m      | 2.80, br t (6.5) | 2.80, dd (5.5, 9.0) |
| 18       | 5.65, dd (15.5, 7.2) | 5.82, ddd (1.5, 8.8, 15.4) | 5.66, dd (7.7, 15.4) | 5.65, dd (7.7, 15.6) | 5.65, dt (9.7, 16.9) |
| 19       | 5.64, m         | 5.76, dd (3.9, 15.4) | 5.67, dd (5.8, 15.4) | 5.57, dd (5.9, 15.6) | 5.27, d (10.0) |
|          | 5.21, d (16.9) | 5.21, d (16.9) | 5.21, d (16.9) | 5.21, d (16.9) | 5.21, d (16.9) |
| 20       | 4.16, t (4.8)   | 4.19, m         | 4.16, m         | 4.20, t (6.3)   | 3.22, dd (3.1, 9.7) |
| 21       | 4.30, m         | 4.27, m         | 4.16, m         | 4.07, quad (6.9) | 4.28, d (6.0) |
| 22       | 1.55, m         | 1.59, m         | 1.59, m         | 1.59, m         | 1.53, dd (1.2, 5.9, 13.7) |
| 23       | 2.39, m         | 2.39, m         | 2.39, q (6.5)   | 2.40, dt (6.6, 12.6) | 2.39, dd (6.6, 8.2, 14.0) |
| 24       | 3.94, sext (6.3) | 4.07, m         | 4.22, m         | 4.26, sext (7.4) | 3.96, m         |
|          | 1.34, d (6.2)   | 1.34, d (6.1)   | 1.32, d (6.3)   | 1.32, d (6.2)   | 1.34, d (6.2)   |

Compound 5 has a terminal methylene group (at $\delta_C$ 119.6, $\delta_H$ 5.27 and $\delta_H$ 5.21) while the tetrahydrofuran moiety connected to C-19 in 1 is connected to C-14 in 5.

Based on COSY correlations (Figure 5), the sporothriolide moiety was the same in compound 5 as in 1. Moreover, COSY correlations from H-13 to H-19 through H-14 ($\delta_H$ 3.25), H-15 ($\delta_H$ 6.16), H-16 ($\delta_H$ 5.56), H-17 ($\delta_H$ 2.80) and H-18 ($\delta_H$ 5.65) together with HMBC correlation between H-13 and C-3 and H-17 and C-3 formed a cyclohexane fragment like in 1. Finally, by using the COSY correlations from H-24 ($\delta_H$ 3.40) to H-20 ($\delta_H$ 3.22), through H-23 ($\delta_H$ 3.96), H-22 ($\delta_H$ 1.53 and 2.39) and H-21 ($\delta_H$ 4.28) we deduce the tetrahydrofuran moiety. The HMBC correlations between H-20 and C-14 and C-15 allowed us to connect this tetrahydrofuran moiety to the sp² methine C-14.

The absolute configuration of sporochartine E (5) was suggested using ROE correlations compared to the absolute configuration of sporochartine A (1) (Figure 6).

ROE correlations between H-2 and H-5/H-6 in the sporothriolide moiety and the ROE correlation between H-20 and H-21 and between H-23 and H-21 in the tetrahydrofuran moiety indicated a similar to that in 1.

Sporochartine E (5), showed a correlation between H-17 and H-2, like in compounds 1 and 4. H-2 also exhibited a correlation with H-13b but not with H-14. This suggests that C-3 and C-17 has the same relative configuration than 4. For C-14, we observed ROE correlations between H-14 and H-13a, H-13a, and H-17 and H-14 and H-24, suggesting a 3R, 14S, 17S configuration for compound 5.

Based on the structure of sporothriolide and the recently reported trienylfuranol A isolated from Hypoxylon muceum, we suggested a hypothetic biosynthetic pathway of sporochartines, involving a “spiro” Diels-Alderase reaction as shown in Figure 7 (Klas et al., 2015; Byrne et al., 2016). The possibility of a non-enzymatic catalysis was excluded as reported previously (Leman-Loubière et al., 2017).

The cytotoxicity of sporochartines was evaluated on three human cancer cell lines, HCT-116 (human colon carcinoma), PC-3 (prostate cancer cell lines) and MCF-7 (breast cancer cell line). The results presented in Table 3 are highly contrasting but nevertheless clearly indicate the impact of sporochartine conformation on the bioassay results.

Thus, sporochartine C (3) is toxic against the three cell lines with IC₅₀ ranging from 7.2 to 21.5 µM. In contrast, sporochartine A (1) is totally inactive at concentrations higher than 100 µM. This may be due to the substantial difference in the spatial conformation of compounds 1 and 3 (Figure 4).
TABLE 2 | $^{13}$C NMR data for sporochartines A–E (Data acquired in CDCl$_3$ at 125 MHz).

| Position | Sporochartine A | Sporochartine B | Sporochartine C | Sporochartine D | Sporochartine E |
|----------|----------------|----------------|----------------|----------------|----------------|
| 1        | 171.8, C       | 173.1, C       | 172.8, C       | 171.9, C       | 172.7, C       |
| 2        | 50.6, CH       | 47.2, CH       | 47.2, CH       | 50.9, CH       | 49.7, CH       |
| 3        | 51.0, C        | 51.0, C        | 50.9, C        | 49.2, C        | 46.3, C        |
| 4        | 177.0, C       | 178.7, C       | 178.6, C       | 176.2, C       | 175.6, C       |
| 5        | 78.2, CH       | 78.7, CH       | 78.7, CH       | 78.0, CH       | 76.4, CH       |
| 6        | 81.2, CH       | 81.1, CH       | 81.3, CH       | 81.3, CH       | 81.6, CH       |
| 7        | 28.9, CH$_2$   | 29.0, CH$_2$   | 29.0, CH$_2$   | 29.0, CH$_2$   | 29.0, CH$_2$   |
| 8        | 25.5, CH$_2$   | 25.6, CH$_2$   | 25.5, CH$_2$   | 25.5, CH$_2$   | 25.5, CH$_2$   |
| 9        | 29.1, CH$_2$   | 29.1, CH$_2$   | 29.2, CH$_2$   | 29.2, CH$_2$   | 29.1, CH$_2$   |
| 10       | 31.7, CH$_2$   | 31.7, CH$_2$   | 31.8, CH$_2$   | 31.8, CH$_2$   | 31.7, CH$_2$   |
| 11       | 22.7, CH$_2$   | 22.9, CH$_2$   | 22.7, CH$_2$   | 22.7, CH$_2$   | 22.7, CH$_2$   |
| 12       | 14.2, CH$_3$   | 14.3, CH$_3$   | 14.3, CH$_3$   | 14.3, CH$_3$   | 14.3, CH$_3$   |
| 13       | 20.9, CH$_2$   | 27.1, CH$_2$   | 20.9, CH$_2$   | 20.9, CH$_2$   | 20.9, CH$_2$   |
| 14       | 22.7, CH$_2$   | 22.7, CH$_2$   | 22.6, CH$_2$   | 22.6, CH$_2$   | 22.6, CH$_2$   |
| 15       | 129.4, CH      | 130.2, CH      | 129.8, CH      | 130.9, CH      | 130.9, CH      |
| 16       | 124.0, CH      | 124.6, CH      | 123.5, CH      | 124.1, CH      | 124.1, CH      |
| 17       | 45.5, CH       | 46.5, CH       | 43.9, CH       | 46.9, CH       | 46.9, CH       |
| 18       | 131.6, CH      | 130.6, CH      | 129.1, CH      | 136.8, CH      | 136.8, CH      |
| 19       | 131.5, CH      | 134.3, CH      | 134.1, CH      | 119.6, CH      | 119.6, CH      |
| 20       | 83.3, CH       | 84.8, CH       | 84.9, CH       | 87.0, CH       | 87.0, CH       |
| 21       | 74.8, CH       | 77.3, CH       | 77.1, CH       | 72.5, CH       | 72.5, CH       |
| 22       | 42.6, CH$_2$   | 42.3, CH$_2$   | 41.2, CH$_2$   | 43.4, CH$_2$   | 43.4, CH$_2$   |
| 23       | 74.3, CH       | 74.1, CH       | 74.1, CH       | 73.9, CH       | 73.9, CH       |
| 24       | 21.7, CH$_3$   | 22.5, CH$_3$   | 22.5, CH$_3$   | 22.6, CH$_3$   | 22.6, CH$_3$   |

[a]$_D^{25}$: $-57^\circ$ (0.5, CHCl$_3$) $+$72$^\circ$ (1.0, CHCl$_3$) $+$93$^\circ$ (0.27, CHCl$_3$) $-152^\circ$ (0.27, CHCl$_3$) $+$51$^\circ$ (c 0.3, CHCl$_3$)

[M+H]$^+$ HRESIMS: 419.2423, 419.2423, 419.2431, 419.2429, 419.2433

IR: 3,521, 2,929, 2,859, 1,771, 1,452, 1,304, 1,175, 1,019 cm$^{-1}$

FIGURE 3 | COSY and HMBC key connectivities for sporochartines A–D.

The lower IC$_{50}$ values were recorded for different sporochartines and against different cell lines, sporochartine B (2) for MCF-7 (2.28 µM), sporochartine C (3) for HCT-116 (7.2 µM) and sporochartine E (5) for PC-3 (5.96 µM).

Our future efforts will focus on the cytotoxic profile, biosynthesis and synthesis of sporochartines. The cytotoxicity profile reveals a non-cytotoxic sporochartine A (1), a large spectrum cytotoxic sporochartine C (3) and more cell line specific
sporochartines B (2), D (4), and E (5). This finding merits future investigation on the mechanisms of action of these new scaffolds of cytotoxic compounds.

The biosynthesis of sporochartines, and the biosynthesis of its two moieties, sporothriolide and trienylfuranol A are still unknown. This opens new and promising opportunities for the discovery of novel biosynthetic microbial clusters.

Finally, having in hand hundreds of milligrams of sporothriolide, the hemi-synthesis of sporochartines is currently in progress based on a final Diels-Alder connection. The selectivity of the chemical catalysis and the proportion of different isomers will be compared to the microbial counterpart. According to our expertise in biocatalysis-based chemodiversification of natural compounds (Adelin et al., 2011; Martins et al., 2015), sporothriolide will be submitted to a
panel of microorganisms in order to pursue the enrichment of sporothriolide related compounds.

**MATERIALS AND METHODS**

**General Experimental Procedures**

Optical rotations $[\alpha]_D$ were measured using an Anton Paar MCP-300 polarimeter. IR spectra were obtained using a Perkin Elmer BX FT-IR spectrometer. NMR experiments were performed using a Bruker Avance 500 MHz in CDCl$_3$ at room temperature. High-resolution mass spectra were obtained on a Waters LCT Premier XE spectrometer equipped with an ESI-TOF (electrospray-time of flight) by direct infusion of the purified compounds. Preparative HPLC was performed using Waters modules consisting of an autosampler 717, a pump 600, a photodiode array detector 2996 and an evaporative light-scattering detector, ELSD 2420. Prepacked silica gel Redisep columns were used for flash chromatography using a Combiflash-Companion chromatogram (Serlabo, France).

All other chemicals and solvents were purchased from SDS (France).

**Animal Material**

The *Sphaerocladina* sponge was collected on 17 December 2015 from the coast of Tahiti (9°45.421'S–139°08.275'W) at 20 m depth (Leman-Loubière et al., 2017).

**Hypoxylon Identification and Cultivation**

*H. monticulosum* CLL205 was isolated from the sponge *Sphaerocladina* and grown at 28°C on a PDB medium (Potatoes Dextrose Broth, DIFCO). The ITS rDNA gene amplification and sequencing were performed, and submitted to NCBI/BLAST database (GenBank). The primers used for PCR amplification were ITS1 F: CTT GGT CAT TTA GAG GAA GTA A (T$_m$: 55°C) and ITS4: TCC TCC GCT TAT TGA TATGC (T$_m$: 53°C). The GenBank accession number for *H. monticulosum* CLL205 sequence is SUB2477083 25758633.seq KY744359. *H. monticulosum* CLL205 was cultivated in a 2L Erlenmeyer containing 1L of PDB medium (DIFCO) in a rotary shaker at 28°C and 130 rpm.

**Compounds Isolation**

The culture broth was extracted with ethyl acetate (3×500 mL). The solvent was concentrated to dryness in vacuo to afford 430 mg of crude extract. 300 mg were submitted to flash chromatography on a Combiflash Companion using a Redisep 12g silica column, eluted with a heptane-ethyl acetate mixture. After concentration in vacuo, we obtained sporothriolide (30 mg), compound 1 (9 mg), 2 (14 mg), 3 (4 mg), 4 (3 mg), 5 (1 mg).
Cytotoxicity Assays

A tetrazolium dye ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide; MTT]-based colorimetric assay was used to measure the inhibition on the proliferation of various human tumor cell lines HCT-116 (human colon carcinoma), PC-3 (prostate cancer cell lines) and MCF-7 (breast cancer cell line). The tested compounds were formulated in DMSO and added to the cells such that the final DMSO concentration ranged from 1 to 3%. Cells were grown in D-MEM medium supplemented with 10% fetal calf serum (Invitrogen), in the presence of penicillin, streptomycin, and fungizone, and plated in 96-well microplates. After 24 h of growth, cells were treated with target compounds from 100 nM to 10 nM. After 72 h, MTS reagent (Promega) was added, and the absorbance was monitored (490 nm) to measure the inhibition of cell proliferation compared to untreated cells. IC_{50} determination experiments were performed in separate duplicate experiments.

Isolated Compounds

**Sporochartine A (1)**: white needles, M.p. 86.5–87.9°C; [α]_D^{25} –57 (c 0.5, CHCl_3). See Tables 1, 2 for complete ^1H, ^13C NMR and IR data. HRESIMS m/z 419.2433 [M + H]^+ (calcd for C_{24}H_{35}O_{6}, 419.2433).

**Sporochartine B (2)**: white powder; [α]_D^{25} +72 (c 1.0, CHCl_3). See Tables 1, 2 for complete ^1H, ^13C NMR and IR data. HRESIMS m/z 419.2419 [M + H]^+ (calcd for C_{24}H_{35}O_{6}, 419.2433).

**Sporochartine C (3)**: white powder (4 mg); [α]_D^{25} +93 (c 0.27, CHCl_3). See Tables 1, 2 for complete ^1H, ^13C NMR and IR data. HRESIMS m/z 419.2433 [M + H]^+ (calcd for C_{24}H_{35}O_{6}, 419.2433).

**Sporochartine D (4)**: white powder; [α]_D^{25} –152 (c 0.27, CHCl_3). See Tables 1, 2 for complete ^1H, ^13C NMR and IR data. HRESIMS m/z 419.2431 [M + H]^+ (calcd for C_{24}H_{35}O_{6}, 419.2433).

**Sporochartine E** (5): white powder; [α]_D^{25} +51 (c 0.3, CHCl_3). See Tables 1, 2 for complete ^1H, ^13C NMR and IR data. HRESIMS m/z 419.2425 [M + H]^+ (calcd for C_{24}H_{35}O_{6}, 419.2434).

Structural elucidation data are reported in the Supplementary Materials.

ASSOCIATED CONTENT

Detailed 1D and 2D NMR, MS and IR spectra of sporochartines are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR CONTRIBUTIONS

CL-L: microbiology chemistry; GL: chemistry; CD: invertebrate investigation; JO: head of the team and science manager.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2017.00399/full#supplementary-material

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