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Full Paper

Marine Bifunctional Sphingolipids from the Sponge *Oceanapia ramsayi*

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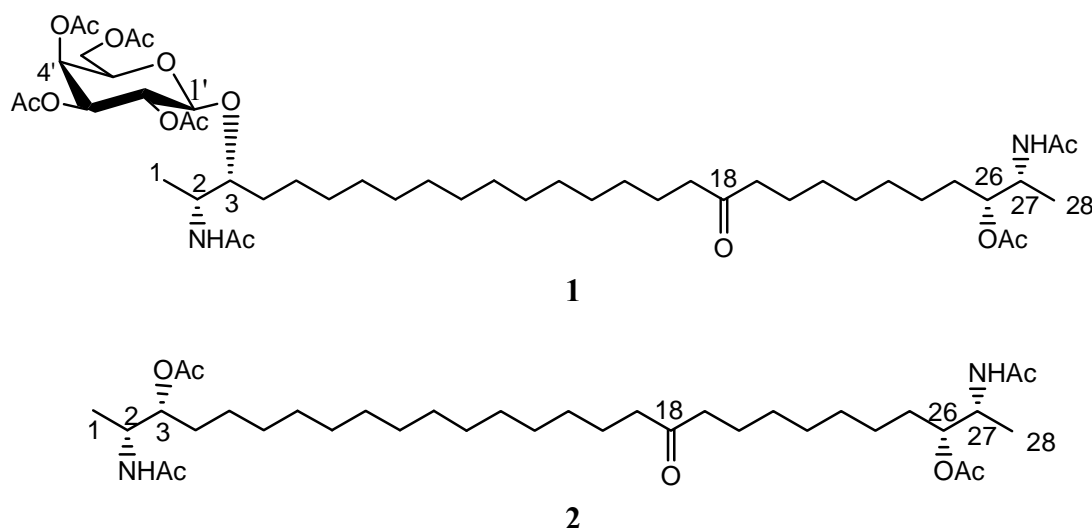
Abstract: During the course of our continuing studies on marine natural lipid products, two known sphingolipids have been isolated for the first time from a specimen of the marine sponge *Oceanapia ramsayi* collected at Itampolo on the west coast of Madagascar in the Indian Ocean. The structures were elucidated using NMR data and by comparison with literature data. The occurrence of these sphingolipids within other *Oceanapia* spp. is discussed.

Keywords: *Oceanapia ramsayi*; Demospongiae, sphingolipids, rhizochalin.

Introduction

The sponge *Oceanapia ramsayi* study has been studied with the aim of discovering new lipid metabolites. Marine sponges belonging to the genus *Oceanapia*, which now includes several other generic names considered as synonyms (*Phloeodictyon* Carter, 1882; *Rhizochalina* Schmidt, 1870; *Biminia* Wiedenmayer, 1977; *Foliolina* Schmidt, 1870 [1]) present a large variety of compounds which may have interesting biological activities, including antifungal properties [2]. Sponges previously classified in *Rhizochalina* present as major components sugar derivatives such as rhizochalin, the first compound isolated from a marine sponge belonging to this genus [3]. Sugar derivatives such as oceanalin A were also recently isolated from *Oceanapia* [2]. Other compound families such as indoles [4], alkaloids [5] or ceramides [6] have also been characterized. During the course of our continuing studies on marine natural lipid products, we have now found in the sponge *Oceanapia ramsayi* two known compounds, rhizochalin and the corresponding aglycone, which were identified as their corresponding peracetates **1** and **2** (Figure 1).

Figure 1. Structures of acetylated compounds **1** and **2**.



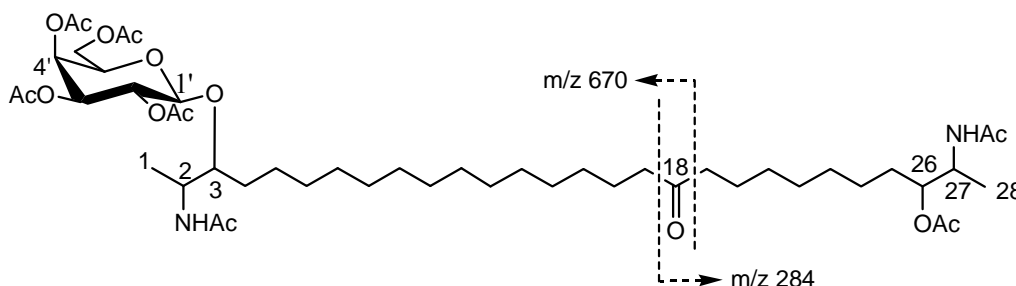
Results and Discussion

The structures of these compounds were determined on the basis of their 1D and 2D NMR spectra and by comparison with literature data [3]. The molecular formula of **1**, isolated as pale yellow oil, was established as $C_{48}H_{82}O_{15}N_2$ on the basis of NMR and EI-MS data (m/z 926, M^+ , calcd 926.5715). The analysis of the corresponding 1H - and ^{13}C -NMR spectra (Table 1) revealed the presence of a signal characteristic of a lipid chain (envelope of CH_2 units) at δ_H 1.23 and δ_C 29.1–29.9 ppm. Moreover, signals at δ_H 4.48 and δ_C 100.4 ppm indicated the presence of an acetal function, suggesting the presence of a sugar. This was confirmed by the chemical shifts of the other carbon atoms at δ 71.0, 69.2, 67.0 and 61.5 ppm along with the respective protons at δ 4.48, 5.04, 3.91, 5.16, 5.39, 4.19 and 4.09 ppm. By comparison with the literature [3, 7], the sugar was identified as a galactose moiety.

Moreover, the coupling constant for the anomeric proton signal H-1' (δ 4.48, $J = 7.9$ Hz) indicated that the galactose had a β -configuration.

The analysis of ^{13}C -NMR and DEPT spectra showed the presence of one methylene group bearing an oxygen (δ 61.3 ppm), two methine groups bearing a nitrogen (δ 47.8 ppm) and a carbonyl carbon (δ 211.7 ppm). HMBC correlation between H-1' and C-3 (δ 82.5 ppm) and COSY correlation between H-1' (δ 4.48 ppm) and H-3 (δ 3.48 ppm) indicated that the galactopyranosyl group was located at C-3. Moreover, the analysis of COSY and HMBC spectra showed also the presence of two NH-acetamides at δ 5.82 ppm (d, $J = 8.8$ Hz) and δ 5.54 ppm (d, $J = 8.9$ Hz), located at C-2 and C-27, respectively. Interpretation of the COSY and HMBC data revealed the presence of two aglycon terminal chains: $-\text{CH}(\text{O})\text{CH}(\text{NHAc})\text{CH}_3$ and $-\text{CH}(\text{OAc})\text{CH}(\text{NHAc})\text{CH}_3$. This was corroborated by the chemical shifts of carbon atoms C-1 (CH_3 , δ 18.5), C-2 (CH-N, δ 46.83), C-3 (CH-O, δ 82.62), C-26 (CH-Oac, δ 76.43), C-27 (CH-N, δ 47.8) and C-28 (CH_3 , δ 18.5) ppm. The HMBC spectrum showed a correlation between the H-19 protons (δ 2.38) and the carbon atom at δ 211.5 ppm, suggesting the presence of a carbonyl group. The analysis of the mass spectrum confirmed the localization of the carbonyl group at C-18, as suggested by the fragmentations on both sides of the ketone function observed at m/z 670, corresponding to $\text{C}_{34}\text{H}_{56}\text{NO}_{12}$, and at m/z 284, corresponding to $\text{C}_{15}\text{H}_{26}\text{NO}_4$ [2, 3] (Figure 2). Moreover, the chemical shifts of the two CH_2 groups (δ_{H} 2.38, t, $J = 7.2$ Hz; δ_{C} 42.83 (C-19) and 42.74 ppm (C-17)) α to the ketone carbon were readily assigned by comparison with the literature data [3]. Thus compound **1** was identified as rhizochalin peracetate.

Figure 2. MS Fragmentation of rhizochalin peracetate **1**.



The structure of compound **2**, obtained as a brown oil, was established by comparison of the 1- and 2-D NMR (Table 2) spectra of **2** with literature data [3, 9] and those of rhizochalin peracetate (**1**). The analysis of the NMR spectrum showed clearly the absence of the sugar seen in compound **1**. Aside from this difference, the NMR spectra are quite close. Again, the MS fragmentations at m/z 382 and m/z 284 indicated the presence of a carbonyl located at C-18. Thus, compound **2** corresponds to the peracetyl aglycone of **1**, in agreement with the literature [3].

This is the first report of these compounds in the sponge *Oceanapia ramsayi*. These compounds have been found previously in other investigated *Oceanapia* spp. (or in its synonym *Rhizochalina*), such as *O. incrustata* [3], *O. bartschi* [4], *O. philipensis* [9], as well as an unidentified *Oceanapia* spp. [2, 5-7]. The common occurrence of these two compounds suggests that they might be good chemotaxonomic markers for this genus.

Sphingolipids seem to play an important role in cellular regulation. These compounds are found within the cell membranes of all living organisms. They have a protection role in the latter against

infection [8]. Two-headed sphingolipids from marine sponges are striking because of their rare α,ω -bifunctionalized structures and high biological activity. Since the discovery of rhizochalin, the first member of this series, only two other compounds have been isolated, which are oceanapiside [9] and the calyxoside [10]. Moreover, each one of these compounds possesses a conserved number of carbon atoms (24) in the long chains between the functionalized termini. It suggests that these compounds are derived from a similar lipid carboxyl precursor.

Table 1. ^1H - (500 MHz) and ^{13}C - (100 MHz) NMR of rhizochalin peracete (**1**) in CDCl_3 ^a.

Position	δ_{C} (mult.)	δ_{H} (mult., <i>J</i> in Hz)
1	18.45 (q)	1.19 (d, 6.7)
2	46.83 (d)	4.09 (m)
3	82.45 (d)	3.48 (m)
4	31.51 (t)	1.58 (m)
5	25.18 (t)	1.57 (m)
6-15	29.09-29.83 (t)	1.23 (brs)
16	23.88 (t)	1.58 (m)
17	42.72 (t)	2.38 (t, 7.2)
18	211.70 (s)	—
19	42.83 (t)	2.38 (t, 7.2)
20	23.41 (t)	1.58 (m)
21-24	29.09-29.83 (t)	1.23 (brs)
25	31.54 (t)	1.58 (m)
26	76.43 (d)	4.85 (m)
27	46.83 (d)	4.21 (ddd, 9.0 ; 4.3; 6.9)
28	18.45 (q)	1.16 (d, 6.7)
NH-C2		5.82 (d, 8.8)
NH-C27		5.54 (d, 8.9)
1'	100.43 (d)	4.48 (d, 7.9)
2'	69.21 (d)	5.16 (dd, 10.5, 7.9)
3'	70.69 (d)	5.04 (dd, 10.1 ; 3.4)
4'	67.94 (d)	5.39 (brd, 3.5)
5'	70.69 (d)	3.91 (t, 6.7)
6'a	61.28 (t)	4.19 (m)
6'b		4.09 (m)

^a in CDCl_3 ; δ ppm. (For Ac, δ_{H} 2.00-2.20; δ_{CO} 169.5-170.4 ppm)

Table 2. ^1H - (500 MHz) and ^{13}C - (100 MHz) NMR of peracetylglucoside 2 ^a.

Position	δ_{C} (mult.)	δ_{H} (mult., <i>J</i> in Hz)
1	19.73 (q)	0.96 (d, 6.7)
2	50.79 (d)	3.97 (m)
3	74.69 (d)	4.74 (m)
4	35.00 (t)	1.41 (m)
5	30.00 (t)	1.41 (m)
6-15	28.50-34.70 (t)	1.22 (brs)
16	28.33 (t)	1.41 (m)
17	46.79 (t)	2.36 (t, 7.1)
18	211.90 (s)	
19	46.79 (t)	2.36 (t, 7.1)
20	28.33 (t)	1.41 (m)
21-24	28.50-34.70 (t)	1.22 (brs)
25	35.00 (t)	1.41 (m)
26	74.69 (d)	4.74 (m)
27	51.34 (d)	3.97 (m)
28	19.73 (q)	0.96 (d, 6.7)
NH-C2		7.69 (d, 8.9)
NH-C27		7.50 (d, 7.9)

^a in MeOD; δ ppm. (For Ac, δ_{H} 2.00-2.20; δ_{CO} 169.5-170.4 ppm)

The absolute configurations of rhizochalin and its aglycone (*threo*, *threo*) differ from those obtained for calyxoside and oceanapiside (*erythro*, *threo*) as well as those obtained for the sphingolipids with methyl termini such as the fumonisins B1 and B2 described by Branham and Plattner [11]. These authors propose that at least two independent amino acid fatty acyl transferases, or homologous subunits in the same enzyme are operative in the biosynthesis of dimeric sphingolipids in marine sponge, one incorporating alanine with *2R,3R* stereoselectivity and the second incorporating serine with *2S,3S* stereoselectivity [12].

Experimental

General

^1H , ^{13}C , COSY, HSQC and HMBC NMR spectra were recorded on a Bruker ARX-500 instrument using standard Bruker pulse sequences.

Biological Material

A specimen of the sponge *Oceanapia ramsayi* (Lendelfeld, 1888; phylum *Porifera*, class Demospongiae, order Haplosclerida, family Phloeodictyidae) was collected at Itampolo (west coast of

Madagascar Island) in 2005. It is a massive sponge, red in life, which displays numerous fistules. A voucher specimen is deposited in the Museum d'Histoire Naturelle de Marseille (MHNM, n°15830.0).

Extraction and Isolation

The crude extract (1.99 g), obtained by extraction of the sponge with CHCl₃/MeOH (1:1) at room temperature, was chromatographed over a Sephadex (LH-20) column, eluted with *n*-heptane/CHCl₃/MeOH mixtures of increasing polarity. Among the five fractions recovered, fractions 2 and 3, obtained with *n*-heptane/CHCl₃/MeOH (40:35:25), were acetylated in order to afford compounds **1** (20 mg) and **2** (15 mg), respectively.

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Sample Availability: Samples of compounds **1** and **2** are available from the authors.

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