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Reidispongiolide A and B, Two New Potent Cytotoxic Macrolides from the New Caledonian Sponge *Reidispongia coerulea*

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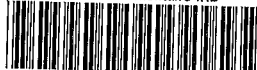
Abstract: Two new 26-membered macrolides, reidispongiolide A (1) and B (2), have been isolated from the New Caledonian marine sponge *Reidispongia coerulea* n.gen. n.sp. Lévi and Lévi and their structures elucidated. They are related to sphinxolides previously isolated from an unknown nudibranch and later from the sponge *Neosiphonia superstes*.

1 and 2 co-occur with sphinxolide B (4) and D (3). These macrolides exhibited potent cytotoxicity against various human carcinoma cells.

The isolation of cytotoxic marine sponge macrolides has been rapidly increasing. They have been isolated from sponges of the genus *Theonella*¹, *Halichondria*², *Mycale*³, *Japsis*⁴ and very recently from a *Spongia* sp.⁵, a *Cinachyra* sp.⁶ and *Hyrtios altum*⁷. Previously we described discovery of potent cytotoxic 26-membered macrolides, sphinxolide⁸ and its congeners sphinxolides B-D, in the New Caledonian sponge *Neosiphonia superstes* (fam. Phymathellidae)⁹.

Continued investigation of New Caledonian marine invertebrates for bioactive metabolites resulted in isolation and structural elucidation of two new cytotoxic macrolides, herein named reidispongiolide A (1) and B (2), from the marine sponge *Reidispongia coerulea* (Demospongiae, Lithistida, Phymathellidae) n.gen. and n.sp.

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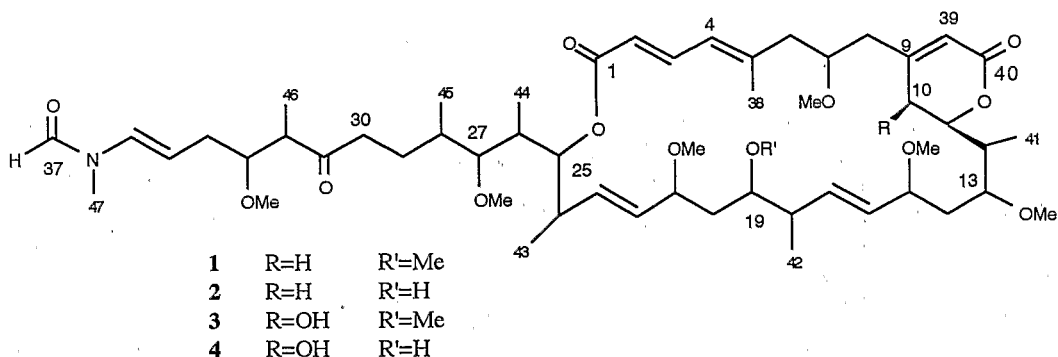
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Lévi and Lévi¹⁰, collected in the South of New Caledonia in the region Banc Eponge sea-mount at a depth of 500 m. These compounds are related to sphinxolides⁹, and co-occur with sphinxolide B (4) and D (3). Like the previous sphinxolides, reidispongiolide A (1) and B (2) are extremely cytotoxic against various human carcinoma cells *in vitro* (Table 1).

RESULTS AND DISCUSSION

The lyophilized sponge (1 Kg) was extracted in Soxhlet with *n*-hexane and CH₂Cl₂, and then at room temperature with CH₂Cl₂:MeOH (8:2) and finally with MeOH. The cytotoxic (*Artemia salina* assay) dichloromethane extract (2.4g) was chromatographed on silica gel (flash column, MeOH/CHCl₃ 0-20%) followed by reverse phase C₁₈ μ -bondapak HPLC with 77% MeOH aq. to give reidispongiolide A (1, 83.1 mg) and sphinxolide D (3, 23.9 mg) and with 75% MeOH aq. to give reidispongiolide B (2, 2.6 mg) and sphinxolide B (4, 4.8 mg).

By means of positive fast atom bombardment mass spectrometry (FABMS), which showed the largest ion peaks at *m/z* 980 (M+Na)⁺ and 958 (M+H)⁺ and by ¹³C NMR data (Table 2), the molecular formula of the major macrolide reidispongiolide A (1), [α]_D²⁰ = -4.8°, was shown to be C₅₄H₈₇O₁₃N, which is 16 mass units less than sphinxolide D (3). ¹H and ¹³C NMR spectra of 1 closely resembled those of sphinxolides and particularly that of sphinxolide D (3). The ¹H NMR (Table 2) spectrum of 1 showed seven methoxyl singlets, six methyl doublets,

Table 1. *In vitro* cytotoxic activity (IC₅₀ in μ g/ml) of reidispongiolide A (1) and B (2)^a.

Tumor cells	1	2	6-mercaptopurine
NSCLC-N6	0.07	0.05	0.76
P388/Dox	0.01	0.02	0.25
P388	0.16	0.06	0.70
KB	0.10	0.06	0.54
HT29	0.04	0.04	0.87

a) NSCLC-N6: human bronchopulmonary non-small-cell-lung-carcinoma. P388: murine leukemia. P388/Dox: murine leukemia expressing the multi-drug resistance gene *mdr*, especially towards doxorubicine. KB: human nasopharyngeal carcinoma. HT29: human colon carcinoma. Results corresponding to the averages of three to five experiments.

one vinylic methyl singlet and, like sphinxolides, restricted rotation about the N-methyl formyl terminus gave rise

Table 2. ^1H and ^{13}C NMR data for reidispongiolide A (1)^{b,c} and B (2)^c (CDCl_3)

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	-	166.7	-	166.7
2	5.79 d (15.3)	120.5	5.82 d (15.3)	120.6
3	7.50 dd (15.3,11.8)	140.2	7.50 (15.3,11.8)	140.2
4	6.00 d (11.8)	126.1	6.00 d (11.8)	125.7
5	-	145.5	-	145.5
6	2.08,2.48 dd	44.5	2.06,2.46 dd	44.4
7	3.45 m	76.9	3.46 m	78.2
8	2.20 dd (3.16,14.7),2.30 m	40.8	2.23 dd (3.16,14.7),2.37 m	40.8
9	-	157.6	-	157.6
10	2.19,2.29	31.2	2.20,2.35	30.6
11	4.22 m	79.3	4.22 m	78.8
12	1.78 dd	39.2	1.92 dd	39.2
13	3.20 m	77.8	3.26 m	78.3
14	1.72,1.89 m	33.4	1.63,1.89 m	34.5
15	3.48 m	79.2	3.50 m	78.8
16	5.18 dd (15.4,8.5)	129.9	5.26 dd (15.4,8.5)	131.0
17	5.52 dd (15.4,7.8)	139.2	5.46 dd (15.4,7.8)	139.0
18	2.32 dd	37.5	2.28 dd	41.2
19	3.08 m	80.6	3.40 m	73.2
20	1.23,1.66 m	36.6	1.45,1.58 m	38.7
21	3.38 m	78.8	3.69 m	79.8
22	5.10 dd	130.3	5.19 dd	131.4
23	5.42 dd (15.1,10.0)	138.6	5.51 dd	137.2
24	2.42 dd	40.6	2.49 dd	40.6
25	5.12 dd	75.3	5.10 dd	75.5
26	1.89 dd	36.4	1.95 dd	36.5
27	2.66 dd	87.1	2.70 dd	87.2
28	1.63 m	34.3	1.68 m	34.4
29	1.32,1.65 m	23.2	1.43,1.71 m	23.3
30	2.45,2.55 m	40.8	2.45,2.52 m	40.8
31	-	213.5	-	213.5
32	2.70 d	49.0 (48.8) ^a	2.72 d	48.9 (49.0) ^a
33	3.41 m	82.2	3.47 m	82.7
34	2.11,2.42 m	30.6 (30.3) ^a	2.16,2.42 m	30.4 (29.8) ^a
35	5.11-5.06 ^a m	105.4 (107.1) ^a	5.11-5.06 ^a m	105.4 (107.1) ^a
36	6.47-7.18 ^a d (15.0)	130.5 (126.3) ^a	6.5-7.18 ^a d (15.0)	130.3 (126.3) ^a
37	8.28-8.02 ^a s	162.1 (160.8) ^a	8.28-8.02 ^a s	162.0 (160.0) ^a
38	1.87 s	17.9	1.90 s	17.8
39	5.83 s	117.4	5.90 s	118.3
40	-	164.0	-	164.1
41	1.10 d (6.9)	9.8	1.10 d (6.9)	11.0
42	0.84 d (6.9)	14.0	0.85 d (6.9)	14.2
43	1.03 d (6.9)	17.4	1.03 d (6.9)	17.8
44	0.91 d (6.9)	9.9	0.91 d (6.9)	9.9
45	0.97 d (6.9)	17.5	0.98 d (6.9)	17.5
46	0.97 d (6.9)	17.5	0.98 d (6.9)	17.5

vs. 156.4 ppm in **3**) and C-39 (117.4 in **1** vs. 119.9 ppm in **3**). Thus the new reidispongiolide A can be defined as the 10-deoxysphinoxolide D (**1**).

Reidispongiolide B (**2**), $[\alpha]_D^{25} = +3.5^\circ$, was smaller than reidispongiolide A (**1**) by a CH_2 unit, FABMS, m/z 966 ($M+\text{Na}$)⁺. Since the ^1H NMR spectrum showed signals for only six methoxyl groups, reidispongiolide B was presumed to be a des-O-methyl reidispongiolide A. Analysis of the COSY spectrum revealed that in C_{10} - C_{25} segment C-10 was a methylene (δ 2.35 and 2.20 ppm) as in **1**, and H-19 was shifted downfield by 0.32 ppm (δ 3.40 in **2** vs. 3.08 ppm in **1**), thus indicating that 19-methoxyl group was replaced by a free hydroxyl group on this carbon. In confirmation, C-19 signal at 80.6 ppm in **1** was replaced by a signal upfield shifted to 73.2 ppm in **2**. A comparison of the spectral data with those of sphinoxolide B (**4**), definitively established the structure of reidispongiolide B as 10-deoxysphinoxolide B (**2**).

Like the other previous sphinoxolides, reidispongiolides, which exist as a mixture of two slowly interconverting isomers, are resistant at attempt of crystallization and their stereochemistry remain to be determined.

EXPERIMENTAL

General Methods.

For general methods see : M. Valeria D'Auria et al., *Tetrahedron*, **1993**, *49*, 8657-8664.

Animal Collection and Preliminary Experiments.

Reidispongia coerulea Lévi and Lévi (Demospongiae, Lithistida, Phymatellidae) was collected during the dredging campaigns (1987, 1989) of the ORSTOM-CNRS, Programme "Substances Marines d'Intérêt Biologique (SMIB)" in the South of New Caledonia (Banc Eponge sea-mount, on the Norfolk Ridge) at depth of 500-515 m. Taxonomic identification was performed by Lévi and Lévi¹⁰ at the ORSTOM Centre de Nouméa where reference specimens are on file (reference 1407). Preliminary assays for cytotoxic (KB cells and P388 leukemia

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