Pergamon

Tetrahedron Vol. 50, No. 16, pp. 4829-4834, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4020/94 $6.00+0.00

0040-4020(94)E0139-K

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

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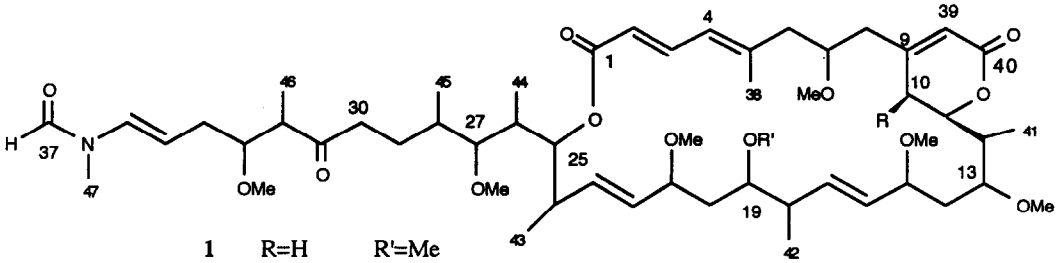
Abstract: Two new 26-membered macrolides, reidispongiolide A (**1**) and B (**2**), have been isolated from the New Caledonian marine sponge *Reidispongia coerulea* n.gen. n.sp. Lévi and Lévi and their structures elucidated. They are related to sphinxolides previously isolated from an unknown nudibranch and later from the sponge *Neosiphonia superstes*. **1** and **2** co-occurr 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, sphinxolide[8] 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, Phymatellidae) n.gen. and n.sp.



1	R=H	R'=Me
2	R=H	R'=H
3	R=OH	R'=Me
4	R=OH	R'=H

Lévi and Lévi[10], 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 sphinxolides[9], and co-occurr 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 CH_2Cl_2, and then at room temperature with CH_2Cl_2:MeOH (8:2) and finally with MeOH. The cytotoxic (*Artemia salina* assay) dicloromethane extract (2.4g) was chromatographed on silica gel (flash column, MeOH/$CHCl_3$ 0-20%) followed by reverse phase C_{18} 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 ^{13}C NMR data (Table 2), the molecular formula of the major macrolide reidispongiolide A (**1**), $[\alpha]_D$=-4.8°, was shown to be $C_{54}H_{87}O_{13}N$, which is 16 mass units less than sphinxolide D (**3**). ^1H and ^{13}C NMR spectra of **1** closely resembled those of sphinxolides and particulary 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 (IC_{50} in μg/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 bronchopulmunary non-small-cell-lung-carcinoma. P388: murine leukemia. P388/Dox : murine leukemia expressing the multi-drug resistance gene mdr, expecially 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 to doubled signals for H-37, N-Me, H-36, H-35 and 33-OMe. Interpretation of 2D-COSY (Fig. 1) disclosed that C_2-C_8, C_{25}-C_{30} and C_{32}-C_{36} portions were those of the 8-demethoxysphinxolides (**3** and **4**) and revealed the ^1H coupling network C_{10}-C_{25} *in* which C-10 was a methylene (δ 2.29 and 2.19 ppm). Thus, reidispongiolide A (**1**) was presumed to be the 10-deoxysphinxolide D. The C-10 signal at 63.0 ppm in **3** was replaced in **1** by a methylene carbon (DEPT) at 31.2 ppm, whereas the C-11 and C-12 signals in **1** were found shifted highfield to 79.3 ppm (82.3 ppm in **3**) and downfield to 39.2 ppm (35.7 ppm in **3**),respectively, as expected for the removal of the hydroxyl at C-10. The remaining signals were almost identical in both spectra apart small variations for the signals of carbons around C-10, *viz.* at C-7 (76.9 in **1** *vs.* 78.0 ppm in **3**), C-8 (40.8 in **1** *vs* 39.2 ppm in **3**), C-9 (157.6 in **1**

Fig.1 Expanded region of 2D-COSY (500 MHz, $CDCl_3$) of reidispongiolide A (**1**)

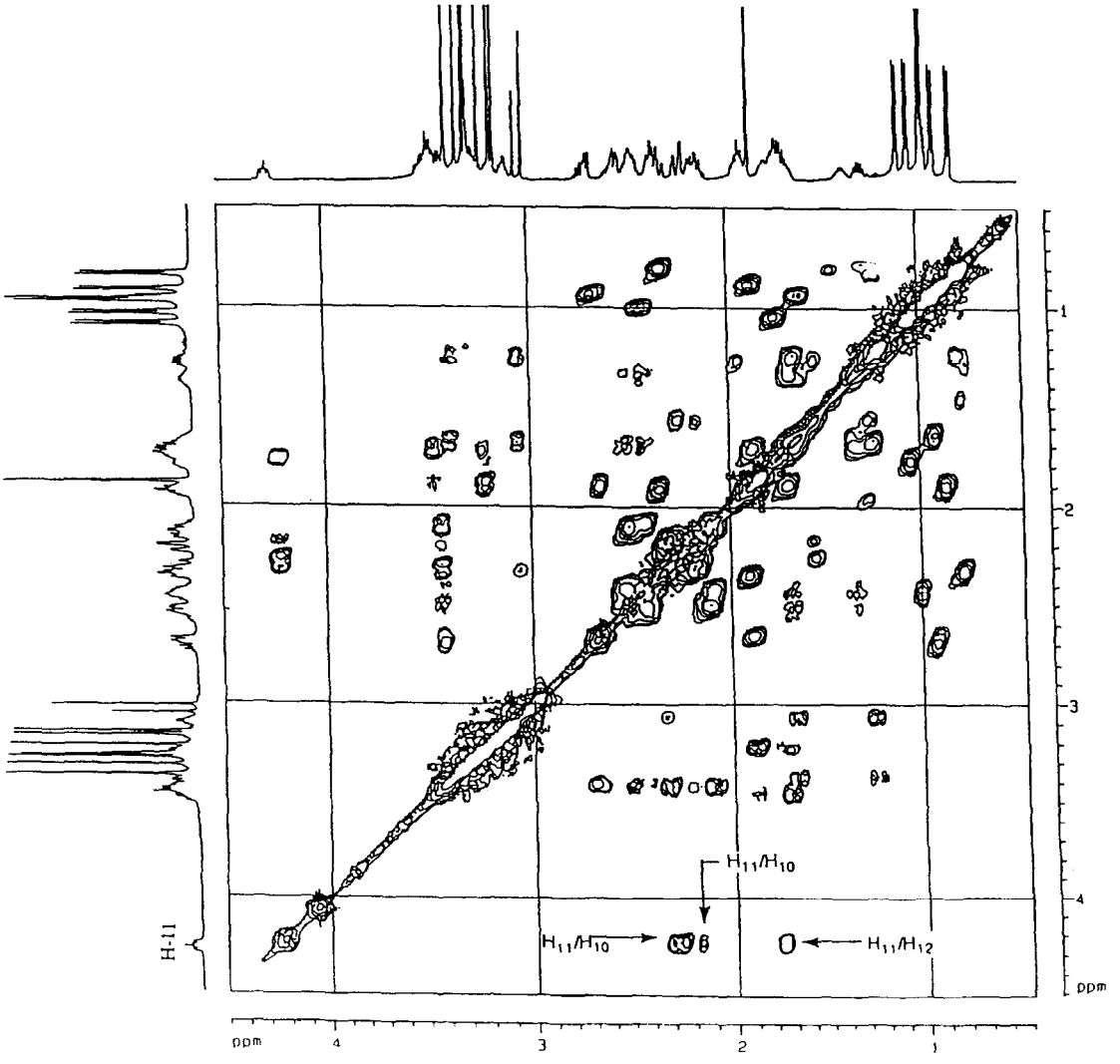


Table 2. ^{1}H and ^{13}C NMR data for reidispongiolide A (1)[b,c] and B (2)[c] ($CDCl_3$)

	1		2	
Position	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)	12.7 (12.8)[a]	0.98 d (6.9)	12.7 (12.8)[a]
47	3.03-3.08[a] s	27.5 (32.9)[a]	3.03-3.08[a] s	27.5 (33.0)[a]
OMe-7	3.26 s	56.8	3.24 s	56.0
OMe-13	3.13* s	55.5*	3.23 s	55.6
OMe-15	3.20* s	55.6*	3.30 s	56.0
OMe-19	3.15 s	57.4	-	-
OMe-21	3.27 s	57.0	3.31 s	56.9
OMe-27	3.30 s	61.5	3.34 s	61.6
OMe-33	3.30-3.25[a] s	57.6	3.36-3.29[a] s	56.8

[a]Signals for minor conformer.The assigments were aided by COSY, HETCOR[b] and DEPT[c] experiments.The coupling constants are given in Hz and enclosed in parentheses.

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-deoxysphinxolide D (**1**).

Reidispongiolide B (**2**), $[\alpha]_D$=+3.5°, was smaller than reidispongiolide A (**1**) by a CH_2 unit, FABMS, *m/z* 966 $(M+Na)^+$. 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 sphinxolide B (**4**), definitively estabilished the structure of reidispongiolide B as 10-deoxysphinxolide B (**2**).

Like the other previous sphinxolides, 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 Experimenis.

Reidispongia coerulea Lévi and Lévi (Demospongiae, Lithistida, Phymatellidae) was collected during the dredging campaigns (1987, 1989) of the ORSTOM-CNRS, Programme "Substances Marines d'Intérest 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 Lévi[10] at the ORSTOM Centre de Nouméa where reference specimens are on file (reference 1407). Preliminary assays for cytotoxic (KB cells and P388 leukemia cells) and antifungal activities (*Fusarium oxysporum, Phythophthora hevea, Penicillium digitatum, Botrytis cinerea, Pyricularia oryzae* and *Helminthosporium sativa*) showed a marked activity of chloroformic extract.

Extraction.

The organisms were freeze dried and the lyophilized material (1 Kg) was extracted in Soxhlet with *n* -hexane and CH_2Cl_2, then with CH_2Cl_2:MeOH 8:2 (3x1L) and with MeOH (3L) at room temperature. The dichloromethane extract was filtered and concentrated under reduced pressure to give 2.4 g of a yellow cytotoxic oil (*Artemia salina* assay).

Isolation.

The crude dichloromethane extract was chromatographed by MPLC on a SiO_2 column (50 g) using a solvent gradient system from $CHCl_3$ to $CHCl_3$:MeOH 8:2. Fractions eluted with $CHCl_3$:MeOH 99:1 (340mg) were further purified by HPLC on a Waters C-18 μ-Bondapak column (7.8 mm i.d. x 30 cm) with 77% MeOH aq. to give 83.1 mg of reidispongiolide A (**1**, t_r=12.8 min.) and 23.9 mg of sphinxolide D (**3**, t_r=18.0 min) and with 75% MeOH aq. to give 2.6 mg of reidispongiolide B (**2**, t_r=19.6 min.) and 4.8 mg of sphinxolide B (**4**, t_r=13.2).

Compound **1** *m/z* 980 $(M+Na)^+$; $[\alpha]_D$=-4.8°

Compound **2** *m/z* 966 $(M+Na)^+$; $[\alpha]_D$=+3.5°

Compound **3** *m/z* 996 $(M+Na)^+$; $[\alpha]_D$=-3.2°

Compound **4** *m/z* 982 $(M+Na)^+$; $[\alpha]_D$=+2.8°

Determination of biological activity.

For cytotoxic assays see : M.V. D'Auria et al. *Tetrahedron* **1993**, *49* , 8657-8664.

ACKNOWLEDGEMENTS

This contribution is part of the project SMIB "Substances Marines d'Intérest Biologique" ORSTOM-CNRS, Nouméa, New Caledonia. The chemical work was supported by "P. F. Chimica Fine 2" CNR, Rome.

We also thank Professor C. Lévi (Muséum Nationel d'Histoire Naturelle, Paris, France) for the identification of the sponge.

Mass spectra were provided by Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli.

The assistance of the staff is gratefully acknowledged.

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(*Received in UK* 14 *December* 1993; *revised* 1 *February* 1994; *accepted* 4 *February* 1994)