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Sphinxolides E-G and Reidispongiolide C: Four New Cytotoxic Macrolides from the New Caledonian Lithistida Sponges *N. superstes* and *R. coerulea*°

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Dedicated to the memory of Professor Giacomino Randazzo

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Abstract. Four new macrolides (**7-10**), structurally related to known sphinxolides (**1-4**) and reidispongiolides (**5-6**), have been isolated following a reinvestigation of *Neosiphonia superstes* and *Reidispongia coerulea*, two Lithistida sponges collected in the deep waters off New Caledonia. In this paper we report their structure elucidation based on spectral data (mainly 2D-NMR and MS) as well as their cytotoxicity determined on a panel of 60 human cancer cell lines (NCI *in vitro* primary screen).
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Key words. Marine sponge; *Neosiphonia superstes.*; *Reidispongia coerulea*; Macrolides; Cytotoxic; 2D-NMR.

INTRODUCTION

In the context of our search for new anti-tumour compounds from New Caledonian marine invertebrates, we had the opportunity to investigate two lithistida sponges, *Neosiphonia superstes* and *Reidispongia coerulea*, whose extracts showed marked activity in cytotoxicity tests against P388, P388dox and KB tumor cell lines. Along with sphinxolide (**1**), previously reported from an unidentified pacific nudibranch,[1] sphinxolides B-D (**2-4**) were first isolated from *Neosiphonia superstes*.[2] Investigation of *Reidispongia coerulea* resulted in the isolation of two macrolides, named reidispongiolides A (**5**) and B (**6**), that are related to sphinxolides and co-occur with sphinxolide B and D.[3] Sphinxolides and reidispongiolides, closely related from a structural point of view, are characterized by the same side chain and a very similar 26-membered macrolactone ring. They differ in that sphinxolides are hydroxylated at C10 position, whereas all derivatives belonging to the reidispongiolide family are not. Sphinxolides and reidispongiolides were found to be potent cytotoxins and recent pharmacological studies demonstrated that the cytotoxicity of sphinxolides is associated with cell cycle arrest in G_2-M phase and induction of apoptosis.[4] Like scytophycins and cytochalasins, sphinxolides have been judged potent antimitotic compounds, causing rapid loss of microfilaments in cultured cells. Moreover, they circumvent multidrug resistance, mediated by overexpression of P-glycoprotein. Therefore, these componds may be useful in the development of new agents for drug-resistant tumors.

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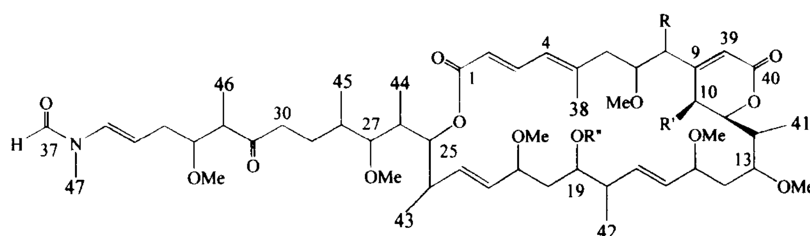
During a recent reinvestigation of *Neosiphonia superstes* and *Reidispongia coerulea*, intended to obtain additional quantities of sphinxolides and reidispongiolides for further biological and stereochemical studies, we isolated four new related compounds named sphinxolides E-G (**7-9**) and reidispongiolide C (**10**). This paper deals with the isolation, structure determination and biological activity of these new molecules.

RESULTS AND DISCUSSION

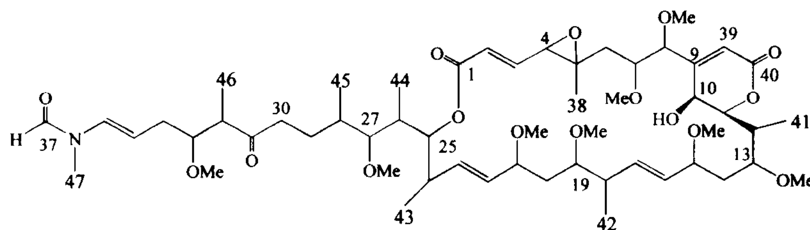
The lyophilized sponge *Neosiphonia superstes* was extracted in a Soxhlet apparatus with *n*-hexane and CH_2Cl_2. The cytotoxic CH_2Cl_2 extract (100% of cell death at a 10 μg/ml dose) was chromatographated on Si-gel followed by reverse HPLC, to give the major compound sphinxolide (**1**) together with spinxolides B-D (**2-4**) and two new congeneric minor macrolides, named sphinxolides E-F (**7-8**).



	R	R'	R''
1	OMe	OH	H
2	H	OH	H
3	OMe	OH	Me
4	H	OH	Me
5	H	H	Me
6	H	H	H

From the CH_2Cl_2 extract of *Reidispongia coerulea*, by following the same isolation procedure (see Exerimental Section), we isolated the reidispongiolides A (**5**) and B (**6**) together with sphinxolides B (**2**) and D (**4**)[3] and two very minor metabolites which we named sphinxolide G (**9**) and reidispongiolide C (**10**).

Sphinxolide E (**7**) showed an intense ion peak at *m/z* 1042.6054 $[(M+Na)^+, \Delta - 2.5$ mmu] in the postive HRFABMS spectrum, 16 mass units higher than that observed for sphinxolide C (**3**). As already reported for a



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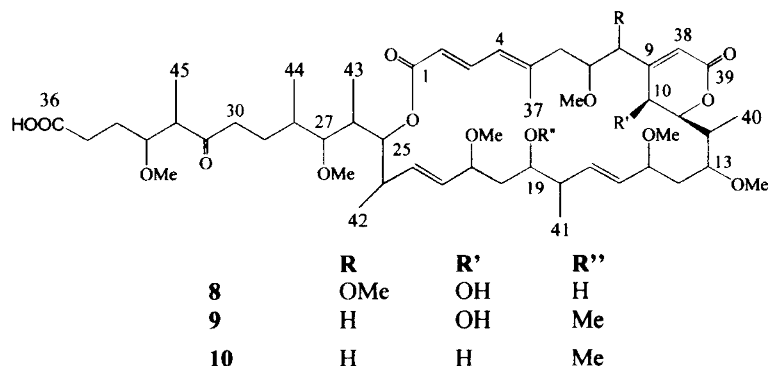
number of marine macrolides containing a N-methyl-formyl end group, such as stylocheilamide,[5] scytophycins,[6] ulapualides,[7] kabiramides,[8] halichondramides[9] and sphinxolides themselves,[2] sphinxolide E (**7**) also exhibited a double set of ^1H and ^{13}C NMR resonances at the C32-C37 fragment. This phenomenon can be explained by assuming that each of these molecules exists as a mixture of two geometrical isomers generated by the restricted rotation around the N-methyl-formyl terminus and slowly interconverting on the NMR time

scale. Full ^{1}H and ^{13}C NMR assignments of 7 were obtained through careful analysis of 2D-NMR spectra, such as COSY, TOCSY, HMQC and HMBC (Table 1 and 2). The ^{1}H and ^{13}C NMR resonances of 7 were virtually superimposable to those of sphinxolide C (**3**), except for the signals ascribable to the conjugate diene (C1-C5



	R	R'	R''
8	OMe	OH	H
9	H	OH	Me
10	H	H	Me

portion). In particular, the signal at δ_H 6.07, assigned to the olefinic proton at C4 position of sphinxolide C (**3**), was replaced in 7 by an hydroxy-methine proton at δ_H 3.28 (d, J= 5.2 Hz). Both the methine C4 and the quaternary C5 appeared as oxygen-bearing carbons up-field shifted to δ_C 60.2 and 64.0, respectively, suggesting that a 4,5-epoxy functionality was present in the molecule. The presence of an epoxide at these positions, in agreement with the MS data of 7 that required an additional oxygen atom (compared to sphinxolide C), is also reminescent of the antifungal macrolide of bacterial origin, pimaricin.[10] Comparison of UV data of sphinxolide C (**3**) and E (**7**) also supported this conclusion. In facts, the absorption at λ_{max} 280 ($\log\varepsilon$ = 4.1) of **3** was shifted in **7** to λ_{max} 226 ($\log\varepsilon$ = 4.1) in agreement with the hypsochromic effect expected for the replacement of the 4,5 double bond with an epoxide functionality. Structural assignment of this portion of the molecule was then completed with the placement of the C38 methyl group (δ_H 1.28, δ_C 17.7) at the C5 quaternary carbon, as indicated by several HMBC correlations around the epoxide ring (Me38/C5, Me38/C4, Me38/C6). Therefore, the structure of sphinxolide E was finally determined as shown in 7. Due to a close similarity in the ^{1}H and ^{13}C NMR spectra of sphinxolide E (**7**) and other known sphinxolides (**1**, **3**), the same relative configuration was assumed around the six-membered δ-lactone ring. However, the complete determination of the stereochemistry of all members of this class remains to be determined and this challenging project (17 stereogenic centers) is currently underway in our laboratories.

Sphinxolide F (**8**) possessed a molecular formula $C_{52}H_{84}O_{16}$ as deduced by HRFABMS data [m/z 963.5657, $(M-H)^-$, Δ - 2.4 mmu]. Inspection of ^{1}H and ^{13}C NMR spectra of sphinxolide F (**8**) clearly showed a single set of resonances. Therefore, from this observation it was readily apparent that **8** lacked the typical N-methyl-formyl end group, responsible for the doubling of selected NMR signals (discussed above). As for **7**, full ^{1}H and ^{13}C NMR assignments were achieved through extensive application of 2D-NMR spectroscopy including DQF-COSY, TOCSY, HMQC and HMBC. A thorough comparison of ^{1}H and ^{13}C NMR data of **8** and sphinxolide (**1**) revealed that both compounds possess the same macrocyclic portion (see Table 1 and 2). As concerning the side chain, NMR data indicated that both **8** and **1** shared the same fragment C26-C33. In addition, proton-proton connectivities arising from analysis of COSY and TOCSY spectra, established that the spin system C32-C36, typically observed for many compounds of this class, was significantly different in **8**. In particular, the H33 signal at δ_H 3.44 was adjacent to two consecutives methylenes (δ_H 1.69 m, 1.90 m, H_2-34; 2.35 dd, H_2-35) giving rise to a $-CH(OCH_3)CH_2CH_2$- moiety. The side chain of **8** could then be completely

determined by placing a carboxyl group at C36 in agreement with the quaternary carbon signal at δ_C 181.3 (C36) and the chemical shift value of H35 at δ_H 2.35. In this view, sphinxolide F (**8**), can be regarded as a truncated derivative of sphinxolide (**1**), arising from oxidation of the C36 aldehyde that is produced upon hydrolysis of the N-methyl-formyl enamine. In order to give additional support to such a structural assignment, we prepared a semisynthetic sample of **8**, by exposing sphinxolide (**1**) to the Jones' reagent (see Experimental Section).

Table 1. ^{1}H NMR data for compounds **7-10** (500 MHz, (d-chloroform).

Position	7	8	9	10
1	-	-	-	-
2	6.01 d (15.6)	5.86 d (15.3)	5.82 d (15.3)	5.80 d (15.3)
3	6.92 dd (15.6, 5.2)	7.52 dd (15.3, 11.8)	7.52 dd (15.3, 11.8)	7.50 dd (15.3, 11.8)
4	3.28 d (5.2)	6.07 d (11.8)	6.05 d (11.8)	6.00 d (11.8)
5	-	-	-	-
6	1.25, 2.17 dd	2.35, 2.50 dd	2.30, 2.43 dd	2.13, 2.53 dd
7	3.74 dd (9.5,1.8)	3.42 m	3.54 m	3.44 m
8	4.03 d (1.8)	3.74 d (6.4)	2.41dd, 2.45 dd	2.24 dd, 2.35 dd
9	-	-	-	-
10	3.78 d (1.8)	4.14 d (1.8)	3.96 d (1.8)	2.20 dd, 2.30 dd
11	4.18 dd (9.5, 1.8)	4.07 dd (9.5, 1.8)	4.09 dd (9.5, 1.8)	4.27 dd
12	2.02 m	2.39 m	2.30 m	1.81 m
13	3.58 m	3.41 m	3.54 m	3.25 m
14	1.87, 2.06 m	1.52, 1.86 m	1.62, 1.89 m	1.75, 1.94 m
15	3.54 m	3.56 m	3.54 m	3.49 m
16	5.28 dd (15.4, 8.5)	5.26 dd (15.4, 8.5)	5.25 dd (15.4, 8.5)	5.20 dd (15.4, 8.5)
17	5.75 dd (15.4, 5.2)	5.43 dd (15.4, 7.8)	5.65 dd (15.4, 7.8)	5.57 dd (15.4, 7.8)
18	2.50 m	2.28 m	2.40 m	2.33 m
19	3.38 m	3.54 m	3.10 m	3.08 m
20	1.26, 1.88 m	1.42, 1.49 m	1.35, 1.75 m	1.25, 1.68 m
21	3.51m	3.64 m	3.45 m	3.40 m
22	5.13 dd	5.14 dd	5.17 dd	5.10 dd
23	5.44 dd (15.1, 10.0)	5.54 dd (15.1, 10.0)	5.47 dd (15.1, 10.0)	5.50 dd (15.1, 10.0)
24	2.45 m	2.44 m	2.44 m	2.46 m
25	5.14 dd	5.13 dd	5.14 dd	5.14 dd
26	1.94 m	1.94 m	1.93 m	1.89 m
27	2.70 dd	2.72 dd	2.69 dd	2.69 dd
28	1.68 m	1.69 m	1.68 m	1.70 m
29	1.37, 1.73 m	1.40, 1.70 m	1.37, 1.68 m	1.32, 1.66 m
30	2.45, 2.57 m	2.46, 2.55 m	2.45, 2.50 m	2.46, 2.53 m
31	-	-	-	-
32	2.72 m	2.72 m	2.73 m	2.72 m
33	3.45 m	3.44 m	3.44 m	3.24 m
34	2.13, 2.45 m	1.69, 1.90 m	1.61, 1.80 m	1.61, 1.80 m
35	5.10-5.08[a] m	2.35, 2.35 dd	2.18, 2.18 dd	2.18, 2.18 dd
36	6.5-7.2[a] d (15.0)	-	-	-
37	8.28-8.02[a] s	1.93 s	1.92 s	1.90 s
38	1.28 s	6.08 s	5.94 s	5.85 s
39	6.06 s	-	-	-
40	-	1.15 d (6.9)	1.14 d (6.9)	1.11 d (6.9)
41	1.16 d (6.9)	0.84 d (6.9)	0.90 d (6.9)	0.84 d (6.9)
42	1.03 d (6.9)	1.02 d (6.9)	1.05 d (6.9)	1.03 d (6.9)
43	1.03 d (6.9)	0.93 d (6.9)	0.94 d (6.9)	0.90 d (6.9)
44	0.90 d (6.9)	0.97 d (6.9)	0.98 d (6.9)	0.97 d (6.9)
45	0.97 d (6.9)	0.98 d (6.9)	0.98 d (6.9)	0.97 d (6.9)
46	0.97 d (6.9)			
47	3.03-3.06[a] s			
OMe-7	3.43 s	3.37 s	3.36 s	3.33 s
OMe-8	3.41 s	3.36 s	-	-
OMe-13	3.33 s	3.24 s	3.31 s	3.29 s
OMe-15	3.25 s	3.25 s	3.25 s	3.22 s
OMe-19	3.15 s	-	3.26 s	3.18 s
OMe-21	3.14 s	3.28 s	3.18 s	3.15 s
OMe-27	3.38 s	3.37 s	3.38 s	3.38 s
OMe-33	3.27-3.23[a] s	3.21 s	3.26 s	3.24 s

[a] Signals for minor conformer. The assigments were aided by 2D-COSY and 2D-TOCSY experiments.

The coupling constants are given in Hz and enclosed in parentheses.

Comparison of spectral data (NMR and MS) of the natural product and the thus prepared truncated derivative of **1** showed that the two compounds were indeed identical. It should be noted that a similar end group with a carboxyl terminus has already been observed for a derivative of halicondramide.[9b]

Sphinxolide G (**9**) is a minor component of the CH_2Cl_2 extract of the sponge *Reidispongia coerulea* and

Table 2. ^{13}C NMR data for compounds **7-10** (125 MHz, (d-chloroform).

Position	7	8	9	10
1	166.1	167.1	166.7	166.8
2	124.2	120.0	120.3	120.4
3	142.8	140.8	140.7	140.4
4	60.2	125.3	125.8	126.0
5	64.0	146.9	145.4	145.5
6	37.4	40.6	43.9	44.4
7	81.0	78.6	77.8	77.8
8	82.8	85.1	39.1	40.9
9	155.8	155.4	156.4	157.5
10	62.9	60.2	62.8	31.2
11	84.1	83.3	82.4	79.4
12	35.3	35.1	35.5	39.2
13	75.8	78.8	78.9	77.8
14	33.8	34.7	34.6	33.4
15	79.6	78.8	79.0	78.9
16	127.2	131.0	130.2	130.0
17	141.8	137.4	138.0	139.1
18	39.8	41.2	38.3	37.5
19	79.8	72.7	81.1	80.6
20	35.9	38.9	36.9	36.6
21	78.9	82.4	79.2	79.4
22	130.7	129.3	130.7	130.6
23	138.0	138.6	138.4	138.5
24	39.5	40.4	40.4	40.6
25	75.8	75.3	75.5	75.3
26	34.5	36.0	36.4	36.5
27	87.0	87.1	87.2	87.2
28	34.5	34.0	34.2	34.4
29	23.2	23.2	23.1	23.1
30	41.1	40.5	40.5	40.7
31	213.5	212	213.5	214.4
32	49.0 (49.1)[a]	49.1	49.1	49.3
33	82.2	82.1	82.3	82.4
34	30.7 (30.5)[a]	26.7	26.7	26.5
35	105.4 (107.1)[a]	32.3	32.3	32.3
36	130.3 (126.3)[a]	181.3	181.3	181.3
37	162.2 (160.8)[a]	18.6	18.0	17.5
38	17.7	121.4	119.7	117.5
39	118.6	164.0	164.3	165.2
40	163.8	11.8	10.6	9.9
41	10.3	15.8	14.4	14.0
42	12.9	17.8	17.9	17.7
43	17.5	9.9	9.9	10.0
44	9.9	17.8	17.3	17.7
45	17.5	15.6	12.5	12.4
46	12.6 (12.7)[a]			
47	27.6 (33.2)[a]			
OMe-7	56.9	57.1	57.3	56.7
OMe-8	59.2	57.8	-	-
OMe-13	56.1	56.7	57.1	56.7
OMe-15	55.6	54.9	55.7	55.4
OMe-19	57.2	-	57.2	56.7
OMe-21	55.7	56.7	55.6	55.3
OMe-27	61.8	60.8	61.4	61.4
OMe-33	57.6	54.7	57.3	57.7

[a] Signals for minor conformer. The assigments were aided by HMQC and HMBC experiments.

co-occurs with reidispongiolide A (**5**). The HRFABMS spectrum showed a pseudomolecular ion peak at *m/z* 947.5712 $(M-H)^-$, (Δ +2.0 mmu), 16 mass units less than sphinxolide F (**8**). ^1H and ^{13}C NMR data of sphinxolide G (**9**) were reminiscent of those of other sphinxolides. Inspection of the ^1H NMR spectrum of **9**

indicated that this compound shared the same oxygenated side chain of sphinxolide F (**8**), whereas modifications were present in both C6-C8 and C18-C21 segments of the macrocyclic portion. Complete assignments of the latter portion of this molecule was performed by careful analysis of TOCSY and HMBC data (see Table 1 and 2), defining the presence of a methylene at C8 position (δ_H 2.41 dd, 2.45 dd), as well as a methoxy functionality at C19. The replacement of the C19-OH in sphinxolide F (**8**) by a O-methyl group in sphinxolide G (**9**) caused small but significative shifts in the ^1H and ^{13}C NMR resonances of the centers around the C19 position, giving definitive support to the proposed structure as shown in **9**.

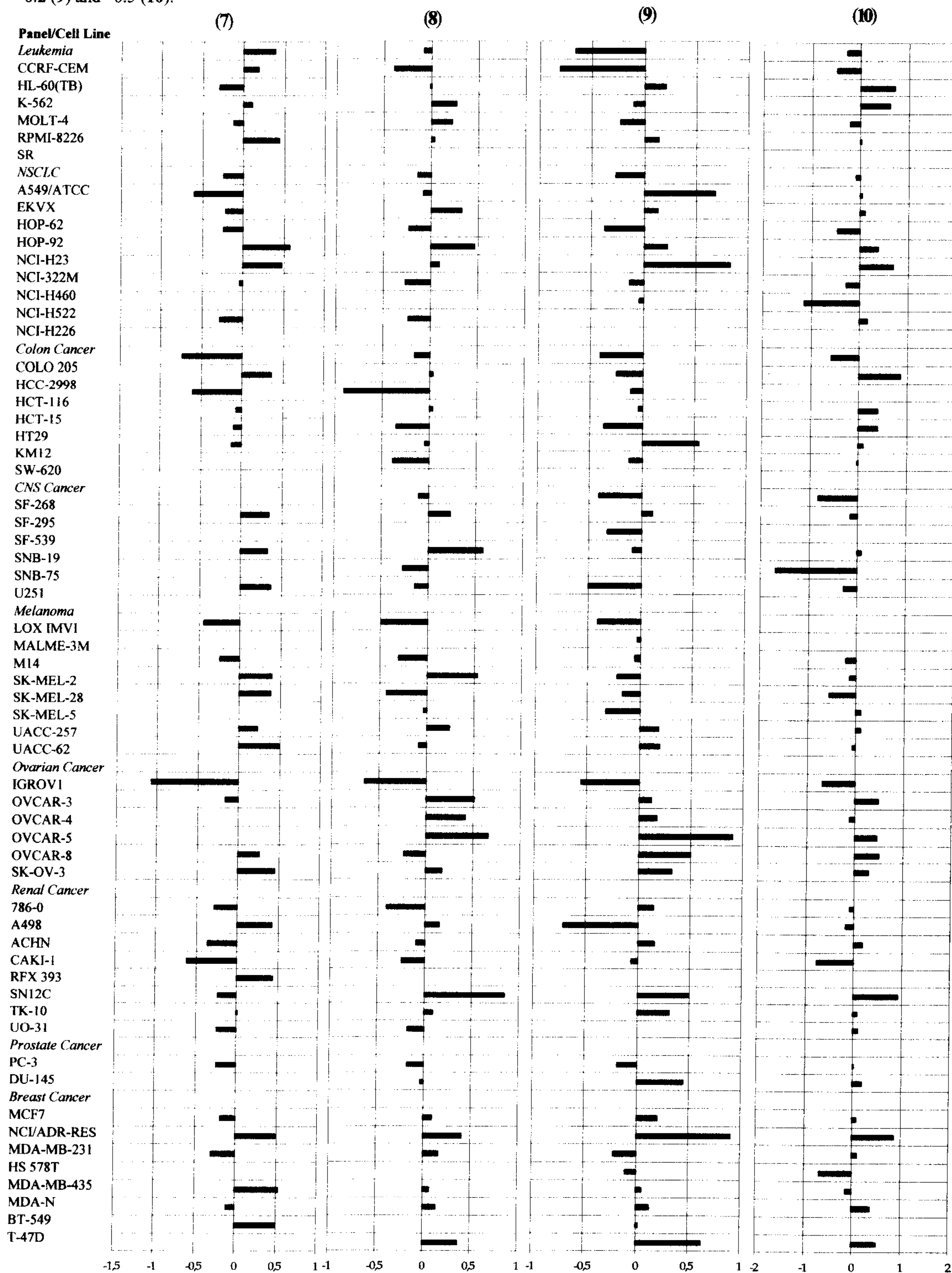
Reidispongiolide C (**10**) gave rise to an intense ion peak at *m/z* 931.5755 [$(M-H)^-$, Δ –2.8 mmu] in the positive HRFABMS spectrum. MS and ^{13}C NMR data indicated that reidispongiolide C possessed one oxygen atom less than sphinxolide G (**9**). Interpretation of COSY and HMQC data revealed that C2-C8, C12-C30 and C32-C36(COOH) portions were indentical with those of acid **9**, while a pertubation was present for the resonances relative to the six membered δ-lactone ring. In particular, proton-proton coupling network C10-C12 revealed that C10 was a methylene (δ_H 2.29dd and 2.19dd). A comparison of NMR data of **10** with that of reidispongiolide A (**5**), the major component of the reidispongiolide family, characterized by the presence of a 10-deoxy-δ-lactone functionality, definitively established the structure of reidispongiolide C (**10**) as 10-deoxy-sphinxolide G.

Biological activity of sphinxolides E-G (7-9) and reidispongiolide C (10)

As stated above, metabolites of both sphinxolide and reidispongiolide families, proved to be very potent and selective cytotoxins. Preliminary cytotoxicity data were obtained for the first compounds of this series (**1-6**) on several tumor cell lines.[2,3] These encouraging results prompted reseachers from the Fox Chase Cancer Center of Philadelphia to undertake an in-depth investigation on their mode of action[4] (see introduction of the present paper).

The newly described compounds (**7-10**) were tested for antiproliferative activity through the US National Cancer Institute's (NCI) human tumor disease oriented *in vitro* primary screen. The panel consists of 60 human tumor cell lines, derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate and breast), against which compounds are tested at a minimum of five concentrations. In this kind of assay, bioactivity data are expressed as molar concentrations that cause inhibition of cell growth (for example: IC_{50} for the 50% inhibition). In this way, one can compare the relative drug concentrations required to produce the same level of biological response in order to assess a measure of the relative cell line sensitivity to a given compound.[11] In order to create a visual means to facilitate this kind of comparison, NCI researchers developed the so called "mean graph". Typical NCI mean graphs (relative to compounds **7-10**) are displayed in Fig. 4. In each of these plots, centered at the arithmetic mean of the logarithm of the IC_{50} values for all cell line responses measured for a given compound, horizontal bars are then projected to the right or to the left of the mean, depending on whether cell line sensitivity to the tested compound is greater or less than average, respectively. The length of a bar is proportional to the difference between the logarithm of the cell line IC_{50} and the mean.
The biological data so gathered for these new macrolides and shown in Fig. 4 would indicate that the truncated derivatives (**8-10**) are less potent by 10-100 times when compared to sphinxolide E (**6**) and other sphinxolides such as **1** or **2**. (data not shown), even though they all seem to display the same degree of cell line selectivity. This finding could be interpreted by assuming that the side chain terminus could play some active role in the

Fig. 4. IC_{50} Mean Graphs from NCI screening of compounds **7-10**. Mean Log IC_{50} (mol/l) for these compounds are: –8.1 (**7**), -7.2 (**8**), -6.2 (**9**) and –6.5 (**10**).



mode of action of these molecules. However, this difference in bioactivity is not very high, suggesting that the macrocyclic portion of these molecules should still have a major part in the drug-receptor recognition process. Analogous data produced for the family of halicondrins,[11] that share with sphinxolides and reidispongiolides similar (intact and truncated) side chains but different macrocyclic moieties, would lead to similar conclusions. Further considerations of the structure-activity relationship (SAR) for these metabolites are complicated by the limitated stereochemical and conformational information currently available.

In conclusion, four new potent antiproliferative macrolides (**7-10**) have been isolated and characterized. The present compounds, along with the related and known sphinxolides and reidispongiolides previously described (**1-6**), may represent useful models for the development of new anticancer agents, also in consideration of their interesting mode of action on the cellular cytoskeleton and their ability to circumvent multidrug tumor resistance (MDR) mediated by overexpression of P-glycoprotein.

EXPERIMENTAL SECTION

General Experimental Procedures. All NMR measurements were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package.

Optical rotation were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Fast atom bombardment mass spectra (FAB MS) were recorded on a VG ZAB instrument (Argon atoms of energy of 2-6 kV) using a glycerol-tioglycerol matrix (with 10μl of a solution of NaCl 0.1 N) in the positive ion mode and in a glycerol-tioglycerol matrix (with triethylamine) in negative ion mode. UV spectra were recorded on a Beckman DU70 spectrophotometer. IR spectroscopy was performed on a IFS 48 Bruker instrument. Reversed-phase HPLC was performed on a C18 μ-Bondapak column (7.8 mm i.d. x 30 cm; flow rate 4.5 ml/min) using a Waters model 6000 A pump equipped with a U6K injector and a differential refractometer, model 401.

Isolation. *Neosiphonia superstes* (class Demospongiae, order Lithistida, family Phymatellidae) was collected during a dredging campaign (1994) of the ORSTOM-CNRS, Programme "Substances Marines d'Intérest Biologique (SMIB)" on the sea mounts of the Norfolk Rise in the South of New Caledonia at a depth of 500-515 m. Taxonomic identification was performed by *Lévi* and *Lévi* at the Museum National d'Histoire Naturelle in Paris and reference specimens are on file in the IRD centre in Nouméa (reference R1408). Preliminary assays for cytotoxic (KB cells and P388 leukemia cells) and antifungal activities (*Fusarium oxysporum*, *Phythophthora hevea*, *Penicillium digitatum*) showed a marked activity of chloroformic extract. The organisms were freeze dried and the lyophilized material (0.5 kg) was extracted with *n*-hexane (3 x 1 l), then with CH_2Cl_2:MeOH 8:2 (3 x 1 l) and with MeOH (3 l) at room temperature. The dichloromethane-methanol extract was filtered and concentrated under reduced pressure to give 2 g of a yellow cytotoxic oil (*Artemia salina*, IC_{50} 10 μg/ml).

The crude dichloromethane-methanol extract was chromatographed by MPLC on a SiO_2 column (50 g) using a solvent system from $CHCl_3$ to $CHCl_3$: MeOH 8:2. Fractions eluted with $CHCl_3$:MeOH 995:5 and $CHCl_3$:MeOH 99:1 (448.9 mg) were further purified by HPLC on a Waters C-18 μ-Bondapak column (7.8 mm i.d. x 30 cm) with MeOH:H_2O (73:27) as eluent (flow rate 5 ml/min) to give 24.4 mg of superstolide A (t_r = 9.8 min.),[12] 7.2 mg of superstolide B (t_r = 23.1 min),[13] 81.1 mg of sphinxolide (**1**) (t_r = 12.0 min), 57.8 mg of sphinxolide B (**2**) (t_r = 15.6 min), 83.3 mg of sphinxolide C (**3**) (t_r = 19.8 min), 69.5 mg sphinxolide D (**4**) (t_r = 21.6 min), 4.7 mg of sphinxolide E (**7**) (t_r = 18.6 min), 5.4 mg of sphinxolide F (**8**) (t_r = 10.0 min.).

Isolation. *Reidispongia coerulea* (Lévi and Lévi, class Demospongiae, order Lithistida, family Phymatellidae) was collected during the same dredging campaign, together with the *Neosiphonia superstes* sample Taxonomic identification was performed by *Lévi* and *Lévi* at the Museum National d'Histoire Naturelle in Paris and reference specimens are on file in the IRD centre in Nouméa (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. The organisms were freeze dried and the lyophilized material (4 kg) was Soxhlet extracted with *n*-hexane (3 x 1 l), then with CH_2Cl_2:MeOH 8:2 (3 x 1l) and finally with MeOH (3 l). The dichloromethane-methanol extract was filtered and concentrated under reduced pressure to give 2.5 g of a yellow cytotoxic oil (100% mortality at a 10 μg/ml dose). The crude dichloromethane-methanol extract was chromatographed by MPLC on a SiO_2 column (50 g) using a solvent system from CH_2Cl_2 to CH_2Cl_2: MeOH 9:1. Fractions eluted with CH_2Cl_2:MeOH 99:1 and CH_2Cl_2:MeOH 98:2 (391.1 mg) were futher purified by HPLC on a Waters C-18 μ-Bondapak column (7.8 mm i.d. x 30 cm) with MeOH:H_2O (77:23) as eluent (flow rate 4.5 ml/min) to give 165.3 mg of reidispongiolide A (**5**) (t_r = 12.8 min) and 57.3 mg of sphinxolide D (**4**) (t_r = 18.0 min), with MeOH:H_2O (70:30) to give 1.5 mg of sphinxolide G (**9**) (t_r = 4 min), with MeOH:H_2O (75:25) to give 18.8 mg of sphinxolide B (**2**) (t_r = 13.2 min), 0.5 mg of reidispongilide B (**6**) (t_r = 19.5 min), 5 mg of reidispongiolide C (**10**) (t_r = 5.6 min).

Compound (**7**): $C_{55}H_{89}NO_{16}$ amorphous powder. $[\alpha]_D^{20}$ + 2.9 (*c* 0.004, $CHCl_3$); IR (KBr): ν_{max} 1716, 1701, 1655,1099 (C-O-C); δ_H (500 MHz, $CDCl_3$) in Table 1;); δ_C (125 MHz, $CDCl_3$) in Table 2; UV/Vis (Methanol): λ_{max} (log ε): 226 nm (4.1); HRMS (FAB positive): MNa^+, found 1042.6054. $C_{55}H_{89}NO_{16}Na$ requires 1042.6079.

Compound (**8**): $C_{52}H_{84}O_{16}$ amorphous powder. $[\alpha]_D^{20}$ + 24.3 (*c* 0.001, $CHCl_3$); IR (KBr): ν_{max} 1720, 1700, 1680; δ_H (500 MHz, $CDCl_3$) in Table 1; δ_C (125 MHz, $CDCl_3$) in Table 2; UV/Vis (Methanol): λ_{max} (log ε): 280 nm (4.0), 208 nm (3.9); HRMS (FAB negative): $M-H^-$, found 963.5657. $C_{52}H_{85}O_{16}$ requires 963.5681.

Compound (**9**): $C_{52}H_{84}O_{15}$ amorphous powder. $[\alpha]_D^{20}$ + 8.0 (*c* 0.004, $CHCl_3$); IR (KBr): ν_{max} 1710, 1700, 1690; δ_H (500 MHz, $CDCl_3$) in Table 1; δ_C (125 MHz, $CDCl_3$) in Table 2; UV/Vis (Methanol) : λ_{max} (log ε): 280 nm (4.0), 212 nm (3.9); HRMS (FAB negative): $M-H^-$, found 947.5712. $C_{52}H_{85}O_{15}$ requires 947.5732.

Compound (**10**): $C_{52}H_{84}O_{14}$ amorphous powder. $[\alpha]_D^{20}$+ 9° (*c* 0.006, $CHCl_3$); IR (KBr): ν_{max} 1725, 1700, 1690; δ_H (500 MHz, $CDCl_3$) in Table 1; δ_C (125 MHz, $CDCl_3$) in Table 2; UV/Vis (Methanol): λ_{max} (log ε): 276 nm (4.1), 212 nm (3.9); HRMS (FAB negative): $M-H^-$, found 931.5755. $C_{52}H_{85}O_{14}$ requires 931.5783.

Conversion of sphinxolide A to sphinxolide F. Sphinxolide A (**1**) (20 mg) was dissolved in dry acetone (300 μl) and cooled to 0 °C, and Jones'reagent (7.7 μl) was added. After being stirred for 2 h under argon, the reaction was quenched with 2-propanol (2 ml). Chloroform (5 ml) and water (5 ml) were added. The organic soluble portion was removed, and the aqueous layer was washed with additional chloroform (2 × 5 ml). The combined chloroform extracts were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* to give a green oil that was purified by HPLC chromatography on a Waters C-18 μ-Bondapak column (7.8 mm i.d. x 30 cm) with MeOH:H_2O (70:30) to obtain the sphinxolide F (**8**) (3.5 mg, 18% of yield) that was identical by ^1H and ^{13}C NMR spectroscopy with the natural product.

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