New Bisindole Alkaloids of the Topsentin and Hamacanthin Classes from the Mediterranean Marine Sponge *Rhaphisia lacazei*

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Chemical investigation of the Et_2O extract of the marine sponge *Rhaphisia lacazei* resulted in the isolation of 13 pure bisindole alkaloids (**1**−**13**). Compounds (**1**−**6**) belong to the class of topsentins and have already been described. Compounds **7**−**13** are new products, closely related to the class of hamacanthins. The major compounds **1**−**3** were tested in vitro for antitumor activity; compounds **2** and **3** showed antiproliferative activity against human broncopulmonary cancer cells (NSCLC-N6) with an IC_{50} of 12 and 6.3 μg/mL, respectively.

Several classes of indole alkaloids have been isolated from a variety of marine invertebrates, including sponges, coelenterates, tunicates, and bryozoans.[1−11] Topsentins, the first examples of brominated bisindole alkaloids showing interesting biological activities, were isolated from the sponge *Topsentia genitrix*.[12,13] Other topsentin-like compounds, differing in the coupling pattern of the indole units were found in several sponges belonging to the genera *Spongosorites*,[14] *Hexadella*, and *Hamacantha*.[15,16] Some of these metabolites showed a wide spectrum of pharmacological activities such as antiviral, cytotoxic, antiinflammatory, and antifungal activities. [10,17,18]

In the course of our continuing study on bioactive metabolites from sponges, we have examined several marine organisms collected in the Mediterranean Sea in the frame of the European project named Bioactive Marine Natural Products in the Field of Antitumoral, Antiviral, and Immunomodulant Activity, financially supported by E. U. In this paper we report the results of chemical and pharmacological investigation of the Mediterranean sponge *Rhaphisia lacazei*.

On the basis of preliminary pharmacological screening, the crude extract of this sponge was selected for chemical analysis. A bioassay-guided fractionation led us to isolate 13 pure bisindole alkaloids (**1**−**13**). Six known compounds (**1**−**6**) possess the topsentin skeleton. The remaining seven (**7**−**13**), all new compounds, are structurally related to the hamacanthins.[16] The major metabolites (**1**−**3**) were tested in vitro on NSCLC-N6 carcinoma cell-line. Only compounds **2** and **3** showed moderate cytotoxic activity (IC_{50}: 12.0 and 6.3 μg/mL, respectively), confirming them to be responsible for the antiproliferative activity of the crude extract. The remaining compounds, not tested because of the small amount obtained, were only chemically investigated.

Results and Discussion

Frozen organisms (1.2 kg wet wt) were minced and extracted with acetone. The aqueous residue, obtained after evaporation of the organic solvent, was then extracted with diethyl ether and *n*-butanol. The diethyl ether extract was sequentially subjected to Si gel chromatography (gradients: petroleum ether to petroleum ether−diethyl ether 30:70 and chloroform to chloroform−methanol 60:40) and reversed-phase HPLC (CH_3CN-H_2O 40:60), affording the known compounds **1**−**6**[12,13] and the new metabolite dihydrohamacanthin B (**7**) as pure compounds. The remaining mixtures were purified by HPLC on a Si gel column ($CH_2Cl_2-CH_3OH$ 96:4), obtaining the pure compounds **8**−**13**, all structurally related to hamacanthins.[16]

Table 1. ^{13}C (150 MHz), ^1H (600 MHz), and HMBC[a] NMR Data for 6″-Deoxybromotopsentin **6** in Acetone-d_6 Containing 1% of CCl_3COOH[b]

	^{13}C	^1H	HMBC (^{13}C)
1			
2	142.1		
3			
4	116.8	8.16 br s	C-2, C-5, C-3′
5	131.7		
1′		11.11 br s	C3′, C3a′
2′	126.6	8.23 d (2.6)	C-5, C-3a′, C-7a′
3′	103.8		
3a′	124.2		
4′	121.2	7.94 d (8.7)	C-3′, C-3a′, C-5′, C-6′, C-7a′
5′	124.1	7.36 dd (8.7−1.5)	C-3a′, C-7′
6′	116.1		
7′	115.5	7.79 d (1.5)	C-3a′, C-5′, C-6′, C-7a′
7a′	138.0		
1″		11.73 br s	C-3″, C-3a
2″	138.1	8.83 d (3.4)	C-3″, C-3a”,C-8″
3″	114.5		
3a″	126.9		
4″	122.1	8.34 dd (6.2−2.0)	C-3″, C-3a″, C-5″, C-6″, C-7a″
5″	123.4	7.33 m	C-3a″, C-7″
6″	124.7	7.34 m	C-4″, C-7a″
7″	113.0	7.63 dd (7.7−1.0)	C-3a″, C-5″, C-7a″
7a″	137.5		
8″	171.7		

[a] Key correlations are shown in bold. [b] *J* values are given in Hz and reported in parentheses. ^1H and ^{13}C signals are referenced to acetone-d_6 at δ 2.07 and 29.5, respectively.

6″-Deoxy-bromotopsentin (**6**),[19] a yellow solid, gave a molecular ion cluster in the EIMS at *m*/*z* 404, 406 [M^+] (ratio 1:1), characteristic of a monobrominated compound. The molecular formula $C_{20}H_{13}BrN_4O$ was deduced by combining mass spectral data and NMR. A careful inspection of ^1H and ^{13}C NMR spectra with those reported for known topsentins[12,13] indicated that this metabolite possesses the same imidazolylbis(indole) unit. The ^1H NMR spectrum recorded in acetone-d_6 showed doubling of all signals, but the doubling could be eliminated by addition of 1% CCl_3COOH to the deuterated solvent, allowing an easier analysis. The spectrum showed the presence of four aromatic spin systems (H-4′ through H-7′ and H-4″ through H-7″; H-1′/H-2′ and H-1”/H-2″) and an aromatic singlet (H-4) accounting for a deoxy-bromotopsentin skeleton. The signal doubling, due to the tautomeric exchange on the

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Table 2. ^{1}H (600 MHz) NMR Data for **7**−**13** in Acetone-d_6[a]

H	7	8	9	10	11	12	13
1	7.02 d (3.8)	6.95 d (3.9)	6.95 d (3.9)	7.07 br s	6.96 br s	6.89 br s	6.86 br s
3	5.06 br s	5.06 s	5.06 s	4.93 s	4.91 s	4.92 s	4.91 s
5	4.71 dd (3.8, 11.0)	4.72 dd (3.9, 11.0)	4.72 dd (3.9, 11.0)	3.20 dd (13.2, 7.0)	3.23 dd (12.3, 8.8)	3.23 dd (13.0, 8.8)	3.23 dd (12.8, 8.8)
			3.29 dd (13.2, 4.8)	3.45 dd (12.3, 4.4)	3.46 dd (13.0, 4.5)	3.46 dd (12.8, 4.4)	
6	3.58 dt (3.8, 11.0)	3.59 dt (3.9, 11.0)	3.59 dd (3.9, 11.0)	5.10 dd (4.8, 7.0)	5.19 dd (4.4, 8.8)	5.19 dd (4.5, 8.8)	5.19 dd (4.4, 8.8)
	3.78 t (11.0)	3.79 t (11.0)	3.79 t (11.0)				
1′	10.23 br s	10.05 br s	10.23 br s	10.31 br s	10.28 br s	10.10 br s	10.28 br s
2′	7.42 d (2.4)	7.39 d (2.2)	7.42 d (2.4)	7.43 d (2.2)	7.41 d (2.2)	7.38 d (2.2)	7.42 d (2.2)
4′	7.83 d (8.5)	7.88 dd (1.3, 8.3)	7.83 d (8.5)	7.86 d (8.4)	7.81 d (8.4)	7.85 d (7.9)	7.82 d (8.5)
5′	7.17 dd (1.5, 8.5)	7.02 br t (8.3)	7.17 dd (1.5, 8.5)	7.13 dd (8.4, 1.3)	7.16 dd (8.4, 1.3)	7.02 br t (7.9)	7.16 dd (8.5, 1.3)
6′		7.09 bt (8.3)				7.11 br t (7.9)	
7′	7.58 d (1.5)	7.38 dd (1.3, 8.3)	7.58 d (1.5)	7.60 d (1.3)	7.61 d (1.3)	7.40 d (7.9)	7.61 d (1.3)
1″	10.38 br s	10.38 br s	10.20 br s	10.38 br s	10.45 br s	10.45 br s	10.28 br s
2″	7.46 d (2.4)	7.47 d (2.5)	7.44 d (2.2)	7.42 d (2.2)	7.49 d (2.2)	7.50 d (2.2)	7.45 d (2.2)
4″	7.87 d (8.5)	7.89 d (8.5)	7.94 d (7.9)	7.57 d (8.4)	7.72 d (8.4)	7.72 d (8.4)	7.75 d (7.9)
5″	7.19 dd (1.6, 8.5)	7.19 dd (1.8, 8.5)	7.04 br t (7.9)	7.13 dd (8.4, 1.3)	7.23 dd (8.4, 1.3)	7.23 dd (8.4, 1.3)	7.08 br t (7.9)
6″			7.20 br t (7.9)				7.17 br t (7.9)
7″	7.63 d (1.6)	7.63 d (1.8)	7.43 br d (7.9)	7.62 d (1.3)	7.67 d (1.3)	7.67 d (7.9)	7.46 br d (7.9)

[a] *J* values are given in Hz and reported in parentheses. Signals are referenced to acetone-d_6 at δ 2.07.

imidazolic ring, was previously reported for topsentins A, B1, and B2.

Further support for structure **6** came from ^{13}C NMR data, which contained 10 quaternary carbons and 10 CH sp^2 resonances in the downfield region (Table 1). Complete assignment of chemical connectivities were obtained by analysis of HSQC and HMBC NMR spectral data (Table 1). In particular, the correlations between H-2′/C-5, H-4/C-3′, H-4/C-2, and H-2″/C-8″ (boldface in Table 1) confirmed the connection of the 3′-bromoindolyl residue to C-5 and of the 3″-indolcarbonyl residue to C-2 positions of the central imidazole ring. This structural hypothesis was fully supported by the fragmentation pattern observed in the EIMS spectrum (*m/z* 287, 289) [M^+ − indole ring − H], *m/z* 261, 263 [M^+ − indolecarbonyl residue + H] and *m/z* 144.

The EIMS spectrum of *cis*-3,4-dihydrohamacanthin B (**7**) revealed an isotopic molecular ion cluster (ratio 1:2:1) at *m/z* 486, 488, 490 [M^+] diagnostic of a dibrominated compound. The composition $C_{20}H_{16}Br_2N_4O$ was deduced by combining MS and NMR data. Analysis of ^{1}H and ^{13}C NMR data (Table 2), along with the comparison of chemical shift values with those of known bromoindoles,[14,17] allowed us to establish the presence of two independent 6-bromoindol-3-yl residues. The remaining substructure, a 3,5-disubstituted piperazin-2-one moiety, was deduced by extensive 1D and 2D NMR experiments (^{1}H,^{13}C, COSY, HSQC, HMBC), along with comparison of spectral data of hamacanthin B.[16] In fact, NMR spectra of **7** and hamacanthin B contained two identical aromatic spin systems accounting for 6-bromoindolyl residues, and a similar aliphatic pattern, assigned respectively to NH-1, H_2-6, and H-5, consisting of one nitrogen-bearing proton (br d at δ 7.02) coupled with two geminal methylene protons (δ 3.78 and 3.58), which were in turn coupled with a deshielded methine proton (dd at δ 4.71). The major difference observed in the ^{1}H NMR spectrum of **7** was the presence of a broad singlet at δ 5.06, which correlated to an aliphatic carbon at 50 ppm in the HSQC spectrum and was assigned to the C-3 position. Moreover, the significant correlations observed in the HMBC spectrum (H-3 and H_2-6 to C-2; H-1 to C-3 and C-5) fully supported the presence of a piperazin-2-one ring.

Cis relative stereochemistry between C-3 and C-5 was established on the basis of the intense correlation between the protons H-3 and H-5 observed in the ROESY spectrum. The EIMS spectrum of *cis*-6′-debromo-3,4-dihydrohamacanthin B (**8**) showed a monobrominated molecular ion at *m/z* 408,410 [M^+] (ratio 1:1), suggestive of a molecular formula $C_{20}H_{17}BrN_40$. The ^{1}H NMR spectrum of **8** was nearly identical to that of **7**, indicating the presence of the same structural framework, and, according to the presence of a single bromine, a pattern attributable to an unsubstituted indol-3-yl framework was found for one of the indole moieties. Analysis of 2D spectral data [HSQC, HMBC, ROESY] (Table 4) allowed us to locate the indol-3-yl moiety at C-3 position of the piperazin-2-one ring. The most relevant correlations were H-2′/C-3, H-3/C-2′, H-3/C-3′, H-5/C-2″, H-5/C-3″, and H-5/C-3a″. The same relative stereochemistry of **7** was also assigned to **8**, on the basis of ROESY evidences.

Table 3. ^{13}C (150 MHz) NMR Data for **7**−**13** in Acetone-d_6[a]

C	7	8	9	10	11	12	13
2	169.8	169.9	169.8	170.5	170.3	170.3	170.1
3	58.5	58.5	58.4	56.2	57.2	57.4	57.3
5	51.9	51.9	51.9	46.2	49.5	49.7	49.7
6	49.5	49.5	49.7	51.4	51.7	52.0	52.1
2′	125.4	124.3	125.4	125.0	125.0	124.1	125.2
3′	115.8	114.7	115.8	114.6	115.3	114.8	115.3
3a′	126.6	127.4	126.6	126.4	126.4	127.4	126.4
4′	122.6	120.4	122.6	122.3	122.2	120.4	120.5
5′	121.9	118.5	121.9	121.7	121.8	118.6	121.8
6′	114.6	121.3	114.6	114.6	114.6	121.3	114.5
7′	114.3	111.2	114.3	114.7	113.8	111.2	114.1
7a′	138.1	137.3	138.1	138.2	138.1	137.3	138.1
2″	123.8	123.9	123.6	124.2	124.0	124.0	124.0
3″	116.0	116.0	116.4	115.2	115.9	116.0	115.4
3a″	125.7	125.8	125.9	126.5	125.1	125.1	126.0
4″	121.5	121.5	120.0	120.4	120.5	120.7	119.0
5″	122.3	122.3	119.7	121.7	122.2	122.4	119.1
6″	114.9	114.9	122.0	114.8	114.9	114.9	121.9
7″	114.7	114.6	112.1	114.1	114.3	114.6	111.7
7″	138.1	138.1	137.6	138.3	138.4	138.3	137.5

[a] Signals are referenced to acetone-d_6 at 29.5.

Mass spectral data (EIMS *m/z* 408, 410 [M^+], ratio 1:1) established that compound **9**, *cis*-6″-debromo-3,4-dihydrohamacanthin B, was a structural isomer of compound **8**. Based on NMR comparison (Table 2), this compound appeared to have the same 3,5-substituted piperazinone ring of **8** and again indol-3-yl and 6-bromo-indol-3-yl residues. The logical structure **9** was fully supported by a complete analysis of proton−proton scalar and dipolar connectivities and C−H long-range correlations. Relevant data were the correlations observed between H-2′/C-3a′, H-3/C-2′, H-5′/C-3a′, H-7′/C-3a′, H-5/C-2″, H-5/C-3a″, and H-5″/C-3a″.

cis-3,4-Dihydrohamacanthin A (**10**) (EIMS spectrum identical to **7**) is structurally related to hamacanthin A,[16] lacking a double bond at the C-3,4 position of the piperazin-

Table 4. Heteronuclear Long-Range Correlations (HMBC) Observed for **7**−**13** in Acetone-d_6

H	7	8	9	10	11	12	13
1	C-3, C-5	C-3, C-5	C-3, C-5	C-3, C-5	C-3, C-5	C-3, C-5	C-3, C-5
3	C-2, C-5, C-2′, C-3′, C-3a′	C-2, C-5, C-2′, C-3′, C-3a′	C-2, C-5, C-2′, C-3′, C-3a′	C-2, C-5, C-2′, C-3′, C-3a′	C-2, C-5, C-2′, C-3′, C-3a′	C-2, C-5, C-2′, C-3′, C-3a′	C-2, C-5, C-2′, C-3′, C-3a′
5	C-3, C-6, C-2″, C-3″, C-3a″	C-3, C-6, C-2″, C-3″, C-3a″	C-3, C-6, C-2″, C-3″, C-3a″	C-3, C-6	C-3, C-6	C-3, C-6	C-3, C-6
				C-3, C-3″	C-6, C-3″	C-6, C-3″	C-6, C-3″
6	C-2, C-5	C-2, C-5	C-2, C-5	C-5, C-2″	C-5, C-3a″	C-5, C-3a″	C-5, C-3a″
	C-2, C-5, C-3″	C-2, C-5, C-3″	C-2, C-5, C-3″				
1′	C-3′, C-3a′	C-3′, C-3a′	C-3′, C-3a′	C-3′, C-3a′	C-3′, C-3a′	C-3′, C-3a′	C-3′, C-3a′
2′	C-3, C-3′, C-3a′, C-7a′	C-3, C-3′, C-3a′, C-7a′	C-3, C-3′, C-3a′, C-7a′	C-3, C-3a′C-7a′	C-3, C-3a′, C-7a′	C-3, C-3a′, C-7a′	C-3, C-3a′, C-7a′
4′	C-3′, C-3a′, C-5′, C-6′, C-7a′	C-3′, C-3a′, C-6′, C-7a′	C-3′, C-3a′, C-5′, C-6′, C-7a′	C-3′, C-6′, C-7a′	C-3′, C-6′, C-7a′	C-3′, C-6′, C-7a′	C-6′, C-7a′
5′	C-3a′, C-7′	C-3a′, C-7′	C-3a′, C-7′	C-3a′, C-7′	C-3a′, C-7′	C-3a′, C-7′	C-3a′, C-7′
6′	C-4′, C-7a′					C-4′, C-7a′	
7′	C-3a′, C-5′, C-6′	C-3a′, C-5′, C-6′	C-3a′, C-5′, C-6′	C-3a′, C-5′	C-3a′, C-5′	C-3a′, C-5′	C-3a′, C-5′, C-6′
1″	C-3″, C-3a″	C-3″, C-3a″	C-3″, C-3a″	C-3″, C-3a″	C-3″, C-3a″	C-3″, C-3a	C-3″, C-3a
2″	C-3″, C-3a″, C-7a″	C-3″, C-3a″, C-7a″	C-3″, C-3a″, C-7a″	C-3″, C-3a″, C-7a″	C-3″, C-3a″, C-7a″	C-3″, C-3a″, C-7a″	C-3″, C-3a″, C-7a″
4″	C-3″, C-3a″, C-5″, C-6″, C-7a″	C-3″, C-3a″, C-5″, C-6″, C-7a″	C-3″, C-5″, C-6″, C-7a″	C-6″, C-7a″	C-6″, C-7a″	C-6″, C-7a″	C-6″, C-7a″
5″	C-3a″, C-7″	C-3a″, C-7″	C-3a″, C-7″	C-3a″, C-6″, C-7″	C-3a″, C-6″, C-7″	C-3a″, C-6″, C-7″	C-3a″, C-6″, C-7″
6″			C-4″, C-7a″				C-4″, C-7a″
7″	C-3a″, C-5″, C-6″	C-3a″, C-5″, C-6″	C-3a″, C-5″, C-6″	C-3a″, C-5″, C-6″	C-3a″, C-5″, C-6″	C-3a″, C-5″, C-6″	C-3a″, C-5″

2-one ring. The structure elucidation of **10** was thus accomplished by an examination of its spectral properties in direct comparison with those of hamacanthin A. Analysis of the ^{1}H NMR spectrum showed the presence of eight aromatic proton signals (H-2′ through H-7′ and H-2″ through H-7″), nearly superimposable with the corresponding signals in the ^{1}H NMR spectrum of hamacanthin A, thus suggesting the presence of two 6-bromo-indolyl residues. The remaining aliphatic proton signals were assigned to a 3,6-disubstituted piperazin-2-one by interpretation of ^{1}H and ^{13}C NMR data (including COSY) and confirmed by long-range C−H correlations. The presence in the ^{1}H NMR spectrum of **10** of an additional proton resonance at δ 4.93, attached to an sp^3 carbon (56.2 ppm), along with the heteronuclear long-range correlations between H-3 and C-2′, C-3′, and C-3a′ (Table 4) fully supported structure **10**. The cis configuration indicated in **10** was established on the basis of ROESY spectral data (H-3/H-5a) along with analysis of the two small coupling constants (J = 4.8 and 6.0 Hz) observed beetween H-6 and H_2-5 proton signals on the piperazin-2-one ring (Figure 2), accounting for H−H pseudo-diequatorial and pseudoaxial−equatorial couplings.

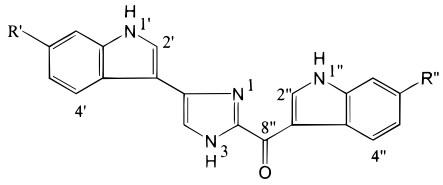
The molecular weight of **11**, *trans*-3,4-dihydrohamacanthin A (*m*/*z* 486, 488, 490) determined by EIMS spectrum, established that **11** is an isomer of **10**. An extensive 1D and 2D NMR study (COSY, HSQC, and HMBC) indicated identical atomic connectivities for both compounds, suggesting that they are indeed stereoisomers. In particular, the relative stereochemistry at position 3 and 6 of the central ring, was established as trans on the basis of a careful analysis of the coupling constants (J = 4.4 and 8.8 Hz) between the H-6 and H_2-5 proton signals (one pseudo-axial−equatorial and one pseudo-diaxial coupling constant) and dipolar correlation H-3/H-5b observed in the ROESY spectrum (Figure 2).

trans-6′-Debromo-3,4-dihydrohamacanthin A (**12**) and *trans*-6″-debromo-3,4-dihydrohamacanthin A (**13**) both showed ion patterns at *m*/*z* 408,410 in their EIMS spectra, identical to **8** and **9**, which were consistent with monobrominated compounds. However, analysis of the NMR data clearly indicated that **12** and **13** have the same structural framework as compound **11**, lacking a bromine at C-6′ and C-6″ indole position, respectively. This hypothesis was fully confirmed after a careful analysis of aromatic spin system connectivities (Table 4). Relevant data were the long-range H−C correlations observed between H-2′/C-3a′, H-5′/C-3a′, H-2″/C-3a″, H-5″/C-3a″, and H-7″/C-3a″ for compound **12**, and H-2′/C-3a′, H5′/C-3a′, H-7′/C-3a′, H-2″/C-3a″, and H-5″/C-3a″ for compound **13**. The relative configuration of the 3- and 6-positions of the central ring, for compound **12** and **13**, was established as trans on the basis of ROESY evidences along with interpretation of coupling constant values between the aliphatic protons on the piperazin-2-one ring (Figure 2), as for compound **11**.

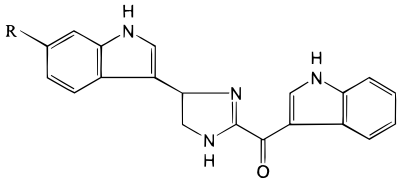
Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker Advance DRX 600 spectrometer. LRMS were recorded on a VG Prospec spectrometer. Optical rotations were determined using a JASCO DIP 1000 polarimeter, using a Na lamp operating at 589 nm. UV spectra were determined using a Beckman DU70 spectrometer. HPLC was performed with a Waters model 510 pump with refractive index detection.

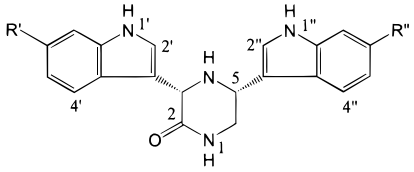
Animal Material. The sponge *R. lacazei* Topsent (Tedaniidae) was collected in September 1994, at −45 m in the "Grotta dei Gamberi", Ustica (Italy). The identification was performed by Prof. M. Sarà, University of Genova; voucher specimens (reference no. US94030) are available in the Dipartimento di



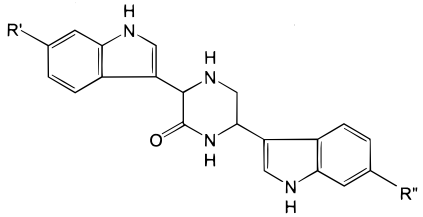
(**1**) Topsentin A R' = H R" = H
(**2**) Topsentin B1 R' = H R" = OH
(**3**) Topsentin B2 R' = Br R" = OH
(**6**) 6"-Deoxy-bromotopsentin R' = Br R" = H



(**4**) 4,5-Dihydro-Deoxy-bromotopsentin
R = Br
(**5**) Topsentin D R = H



(**7**) *cis*-3,4-Dihydrohamacanthin B
R' = Br R" = Br
(**8**) 6'-Debromo- *cis*-3,4-Dihydrohamacanthin B
R' = H R" = Br
(**9**) 6"-Debromo-*cis*-3,4-Dihydrohamacanthin B
R' = Br R" = H



(**10**) *cis*-3,4-Dihydrohamacanthin A
R' = Br R" = Br
(**11**) *trans*-3,4-Dihydrohamacanthin A
R' = Br R" = Br
(**12**) 6'-Debromo-*trans*-3,4-Dihydrohamacanthin A
R' = H R" = Br
(**13**) 6'-Debromo-*trans*-3,4-Dihydrohamacanthin A
R' = Br R" = H

Figure 1. Known (**1**−**6**) and new (**7**−**13**) indole alkaloids from *R. lacazei*.

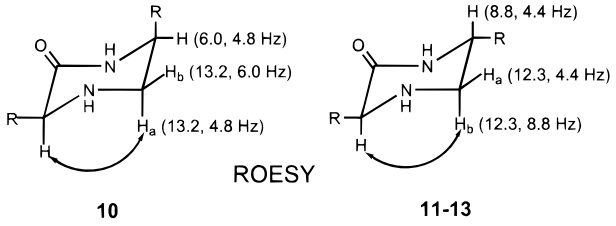


Figure 2. Relative configuration for compounds **10**−**13** via dipolar correlations and coupling-constant studies.

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Extraction and Isolation. Frozen organisms (1.2 kg wet wt) were minced and extracted in acetone (3 × 1.5 L). After concentration, the aqueous residue was extracted with diethyl ether (3 × 1.5 L) and then with *n*-butanol (3 × 1.5 L). A portion (3 g) of the diethyl ether extract (17 g) was fractionated by Si gel chromatography (gradients: petroleum ether to petroleum ether−diethyl ether, 30:70, and chloroform to chloroform−methanol, 60:40). Late eluting fractions were further subjected to reversed-phase HPLC (*μ*-Bondapak C_{18} 8 × 300 mm, eluent CH_3CN-H_2O 40:60, flow rate 5 mL/min) to collect compounds **1**−**7** (15, 18, 20, 1.5, 1.0, 1.0, and 2 mg respectively, all as yellow amorphous solids) as pure samples and also mixtures of **8**−**9**, **10**−**11**, and **12**−**13**. Subsequent purification on Si gel HPLC ($CH_2Cl_2-CH_3OH$ 96:4) provided the pure compounds **8**−**13** (1, 1.2, 2, 2, 0.7, and 1 mg, respectively, all as yellow amorphous solids).

6″-Deoxy-bromotopsentin (6): EIMS *m/z* 406/404 $[M]^+$(92, 92), 326 (20), 290/288 (9, 9), 289/287 (60, 60), 263 (3), 261 (12), 239 (12), 209 (17), 181 (8), 163 (24), 154 (9), 144 (100), 128 (10), 117 (43), 116 (40).

***cis*-3,4-Dihydrohamacanthin B (7):** $[\alpha]^{25}_D$ +98.7 (*c* 0.2, MeOH); EIMS *m/z* 490/488/486 [M](+10,20,10), 475/473/471(10,20,10), 433/431/429(13,26,13), 328 (44), 293/291(54,54), 264/262(28,28), 238 (29), 197/195(100,100).

6′-Debromo-*cis*-3,4-dihydrohamacanthin B (8): $[\alpha]^{25}_D$ +23.2 (*c* 0.1, MeOH); EIMS *m/z* 410/408 $[M]^+$(15,15), 395/393(26,26), 353/351(19,19), 293/291(26,26), 264/262(10,10), 236 (38), 223 (31), 213 (34), 197/195(43,43), 184 (22), 157 (41), 143 (50), 130 (67), 117 (100).

6′-Debromo-3,4-*cis*-dihydrohamacanthin B (9): $[\alpha]^{25}_D$ +52.1 (*c* 0.1, MeOH); EIMS *m/z* 410/408 $[M]^+$(13,13), 395/393(20,20), 353/351(15,15), 293/291(24,24), 264/262(12,12), 236 (32), 223 (31), 213 (30), 197/195(38,38), 184 (18), 157 (38), 143 (53), 130 (62), 117 (100).

***cis*-3,4-Dihydrohamacanthin A (10):** $[\alpha]^{25}_D$ + 8.1 (*c* 0.2, MeOH); EIMS *m/z* 490/488/486 $[M]^+$(5,10,5), 293/291,(80,80), 197/195(90,90).

***trans*-3,4-Dihydrohamacanthin A (11):** $[\alpha]^{25}_D$ + 5.3 (*c* 0.2, MeOH); EIMS *m/z* 490/488/486 $[M]^+$(5,10,5), 293/291,(80,80), 197/195(90,90).

6′-Debromo-*trans*-3,4-dihydrohamacanthin A (12): $[\alpha]^{25}_D$ +4.8 (*c* 0.07, MeOH); EIMS *m/z* 410/408 $[M]^+$(11,11), 293/

291(24,24), 285 (40), 239 (33), 223 (39), 213 (47), 197/195(49,49), 145/143(68,68), 130 (28), 117 (100).

6″-Debromo-*trans*-3,4-dihydrohamacanthin A (13): $[\alpha]^{25}_{D}$ +4.9 (*c* 0.1, MeOH); EIMS *m*/*z* 410/408 $[M]^+$(8,8), 293/291(20,20), 285 (36), 239 (29), 231 (21), 223 (35), 213 (42), 197,195(43,43), 145/143(65,65), 130 (25), 117 (100).

Cytotoxic Assays. Experiments were performed in 96- well microtiter plates ($2 \cdot 10^5$ NSCLC−N6 cells/mL). Cell growth was estimated by a colorimetric assay based on the conversion of tertrazolium dye to a blue formazan product using live mitochondria.[20] Eight determinations were performed for each concentration. Control growth was estimated for 16 determinations. Optical density at 570 nm, corresponding to solubilized formazan, was read for each well on Titertek Multiskan MKII. The IC_{50} values found by this procedure for topsentin B1 and B2 were 12.0 and 6.3 μg/mL, respectively.

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