Tetrahedron Vol. 46, No. 3, pp. 715-720, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

BROMINATED BIS(INDOLE) ALKALOIDS FROM THE MARINE SPONGE *HEXADELLA* SP.

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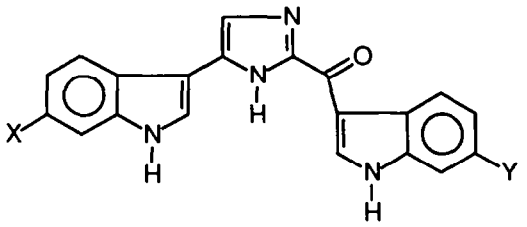
(*Received in USA* 23 *May* 1989)

ABSTRACT *Topsentin C (7), dragmacidon A (8) and dragmacidon B (9), three new brominated bis(indole) alkaloids, have been isolated from the Pacific Ocean sponge Hexadella sp. collected off the coast of British Columbia. The proposed structures for 7, 8 and 9 are based on spectroscopic analysis. Dragmacidon A (8) showed significant cytotoxicity in the L1210 assay.*

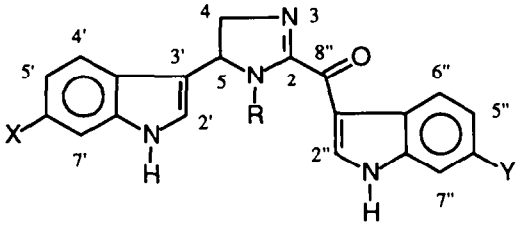
A number of cytotoxic bis(indole) alkaloids have been discovered in marine sponges. The first compounds reported in this series were topsentin (**1**), bromotopsentin (**2**), and deoxytopsentin (**3**)[1] isolated from *Topsentia genitrix* collected off the coast of France.[2] Shortly thereafter, topsentin (**1**), bromotopsentin (**2**), and 4,5-dihydro-6"-deoxybromotopsentin (**4**) were reported from several deep water Caribbean sponges in the genus *Spongosorites*.[3] Another deep water Caribbean sponge, *Dragmacidon* sp., yielded dragmacidon (**5**),[4] a related metabolite that contains a piperazine rather than an imidazole spacer between the indole residues. A completely different type of dimerization was discovered in fascaplysin (**6**), a blood red pigment isolated from the Fijian sponge *Fascaplysinopsis* sp.. Fascaplysin (**6**) has a fully aromatic pentacyclic ring system incorporating a 2,2' bond between the indole residues.[5]

We have been investigating the chemistry of the deep water sponge *Hexadella* sp. collected by manned submersible (-100 to -200m) and SCUBA (-40m) in Jervis Inlet, B.C.. Initially we reported the isolation of bromotopsentin (**2**) as the only metabolite found in the sponge samples collected by submersible and the dibromotyrosine derived metabolites hexadellins A and B as the only metabolites found in the SCUBA collected material.[6] We have subsequently made several much larger collections of *Hexadella* sp. via SCUBA at -40m in Jervis Inlet and we now wish to report the isolation of three new bis(indole) alkaloids, topsentin C (**7**), dragmacidon A (**8**) and dragmacidon B (**9**), as very minor constituents of these sponges.

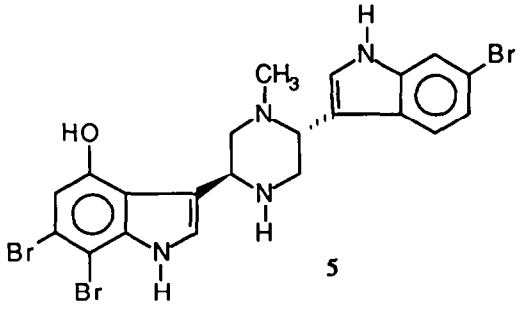
Freshly collected specimens of *Hexadella* sp. (800g wet wt.) were immediately immersed in methanol. The ethyl acetate soluble portion of the methanol extract was fractionated by Sephadex LH20 chromatography ($MeOH/CH_2Cl_2$ 7:3). Early eluting fractions were further subjected to sequential application of silica gel flash (gradient: EtOAc/hexane 7:3 to EtOAc/MeOH 4:1), reverse phase flash (gradient: $MeOH/H_2O$ to MeOH), and radial thin layer (silica gel: EtOAc/MeOH 9:1) chromatographies to give pure samples of dragmacidons A (**8**) (6mg) and B (**9**) (6mg). The late eluting LH20 fractions were further fractionated by radial thin layer chromatography (silica gel: EtOAc/hexane 3:2) to give pure topsentin C (**7**) (3mg).



1 X=H Y=OH
2 X=Br Y=OH
3 X=H Y=H



4 X=Br Y=H R=H
7 X=Br Y=Br R=Me



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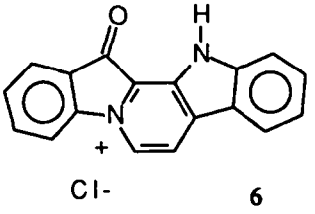

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Topsentin C (**7**), obtained as a yellow solid, gave a parent ion cluster in the EIHRMS at m/z 501.9644/ 499.9674/ 497.9672 Da appropriate for a molecular formula of $C_{21}H_{16}N_4OBr_2$ (ΔM: -0.8/+0.2/-2.0mmu). The ^{1}H nmr spectrum of topsentin C (**7**) contained two sets of resonances that could be assigned to 6-bromoindol-3-yl residues by comparison of their chemical shifts and coupling constants to those reported for 4,5-dihydro-6"-deoxybromotopsentin (**4**) (Table 1). Difference nOe experiments confirmed the attachment of the bromine atoms to position 6 in both indoles. Thus, irradiation of the indole NH resonance at δ 10.34 (H1') induced nOe's in the resonances at δ 7.22 (H2') and 7.63 (H7'), while irradiation of the indole NH resonance at δ 10.71 (H1") induced nOe's in the resonances at δ 8.62 (H2") and 7.66 (H7"). Double resonance and COSY ^{1}H nmr experiments were used to assign the rest of the proton resonances in the two indole rings as indicated in Table 1. The remaining resonances in the ^{1}H spectrum of topsentin C (**7**) could be assigned to a methyl group attached to a nitrogen atom (δ 3.05,s) and to a three spin system consisting of a pair of geminal methylene protons (δ 4.27 and 4.41: H4a, H4b) and an adjacent methine proton (δ 5.16: H5) (Table 1).

A number of pieces of evidence indicated that the two indole rings in topsentin C (**7**) were linked by a 2-keto-4,5-dihydroimidazole spacer similar to the one present in 4,5-dihyro-6"-deoxybromotopsentin (**4**).[3] The downfield shift of the H2" resonance in the ^{1}H nmr spectrum of **7** (Table 1) demanded the attachment of a carbonyl functionality at C3" and the chemical shifts of the carbon resonances assigned to the ketone (C8") and imine (C2) carbons in **7** (δ 157.8 and 158.0: see experimental) were nearly identical to the chemical shifts of the resonances assigned to the corresponding carbon atoms in **4** (δ 159.1 and 160.5).[3] These observations, along with the presence of a carbonyl stretching band at 1643 cm^{-1} in the IR spectrum of **7**, supported the attachment of the 3-ketoindole residue to the imine carbon (C2) of the dihydroimidazole ring. A small scalar coupling observed between H5 (δ 5.16) and H2' (δ 7.22), and nOe's between H4 (δ 4.41) and H7' (δ 7.63) and between H5 (δ

5.16) and H7', confirmed the attachment of the second indole ring to C5 of the dihydroimidazole ring. A strong nOe between H5 (δ 5.16) and the N methyl protons (δ 3.05) located the methyl group on N1. Topsentin C (**7**) is closely related to 4,5-dihydro-6-deoxybromotopsentin (**4**), differing only by the additional bromo substituent at C6" and the methyl group attached to N1.

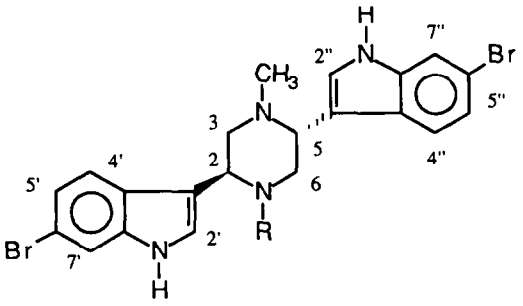
Table 1: ^{1}H NMR data for 4,5-dihydro-6"-deoxybromotopsentin (4) and topsentin C (7).

C#	4[a]	7[b]
4	3.60,ddd(12.1,4.6,4.4)	4.27,dd(16.5,5.3)
	3.45,ddd(12.1,9.5,2.1)	4.41,dd(16.5,5.2)
5	5.23,dd(9.5,4.7)	5.16,ddd(5.3,5.2,<1)
1'NH	11.13,bs	10.34,bs
2'	7.29,bs	7.22,dd(2.5,<1)
4'	7.67,d(8.5)	7.69,d(8.5)
5'	7.12,dd(8.5,1.7)	7.20,dd(8.5,1.7)
7'	7.58,d(1.7)	7.63,d(1.7)
1''NH	11.52,bs	10.71,bs
2''	8.38,bs	8.62,d(2.7)
4''	8.37,ddd(8.0,1.0,0.7)	8.37,d(8.7)
5''	7.02,ddd(8.0,8.0,1.0)	7.20,dd(8.7,1.8)
6''	7.13,ddd(8.0,8.0,1.0)	-
7''	7.42,ddd(8.0,1.0,0.7)	7.66,d(1.8)
N1Me	-	3.05,s

[a] (360MHz) recorded in 1% TFA in Me_2SO-d_6 (see ref. 3)

[b] (400MHz) recorded in acetone-d_6 (δ ppm from internal TMS, multiplicity (J in Hz))

Dragmacidon A (**8**), obtained as a pale yellow powder, gave parent ion clusters at m/z 387/389/491 Da (M + 1) in the CIMS and at 486.0042/488.0027/490.0027 Da (ΔM: -1.4/-0.9/+0.8 mmu) in the EIHRMS corresponding to a molecular formula of $C_{21}H_{20}N_4Br_2$. The ^{1}H nmr spectrum of **8** also contained resonances that could be assigned to two 6-bromoindol-3-yl residues (Table 2). Double resonance and nOe experiments confirmed the indole assignments. A series of six aliphatic methine resonances in the ^{1}H nmr spectrum of **8** were shown by double resonance experiments to belong to two separate three spin systems each consisting of a pair of geminal methylene protons and a neighboring methine proton (δ 2.38, $H3_{ax}$; 3.18, $H3_{eq}$; 4.43, H2 and δ 3.07, $H6_{eq}$; 3.29, $H6_{ax}$; 3.41, H5). The remaining resonance in the ^{1}H nmr spectrum of **8** could be assigned to an N methyl group (δ 2.09,s). Acetylation of **8** gave the monoacetamide **10**, revealing that the final proton required by the molecular formula of **8**, but not apparent in its ^{1}H nmr spectrum, was a NH proton on a secondary amine. The aliphatic region of the ^{13}C nmr spectrum of **8** contained a total of five resonances corresponding to the one N methyl (δ 44.2,q), two methine (δ 63.3,d and 54.1,d), and two methylene (δ 64.2,t and 54.3,t) carbons indicated by the ^{1}H nmr data.



8 R=H
9 R=Me
10 R=Ac

The ^1H nmr chemical shifts and coupling constants and the ^{13}C nmr chemical shifts of the aliphatic resonances assigned to the methine, methylene and N methyl protons and carbons in dragmacidon A (**8**) were nearly identical to those reported for dragmacidon (**5**) (Table 2).[4] Based on this strong similarity, we assigned structure **8** to dragmacidon A. Difference nOe experiments (see experimental) were consistent with the proposed structure and they clearly indicated that both indole residues resided in equatorial orientations.

Table 2. ^1H nmr data (acetone-d_6) for dragmacidon (5), dragmacidon A (8) and dragmacidon B (9).

C#	5[a]	8[b]	9[b]
2	4.35,dd(10.3,2.3)	4.43,dd(10.4,2.6)	3.60,dd(10.6,2.9)
3(ax)	2.39,dd(10.3,11.9)	2.38,dd(10.4,11.0)	2.64,dd(10.9,11.0)
3(eq)	3.05,brm	3.18,dd(11.0,2.6)	2.93,dd(11.0,2.9)
5	3.46,dd(3.9,11.3)	3.41,dd(10.5,3.0)	3.60,dd(10.6,2.9)
6(ax)	3.33,dd(11.3,11.9)	3.29,dd(11.0,10.5)	2.64,dd(10.9,11.0)
6(eq)	3.13,dd(3.9,11.9)	3.07,dd(11.0,3.0)	2.93,dd(11.0,2.9)
1'NH	10.5,bs	10.28,bs	10.29,bs
2'	7.27,s	7.39,d(1.8)	7.38,d(2.3)
4'	-	7.81,d(8.5)	7.92,d(8.5)
5'	6.70,s	7.17,dd(8.5,1.2)	7.17,dd(8.5,1.7)
7'	-	7.61,d(1.2)	7.62,d(1.7)
1''NH	10.8,bs	10.28,bs	10.29,bs
2''	7.36,d(1.8)	7.35,d(2.3)	7.38,d(2.3)
4''	7.84,d(8.6)	7.91,d(8.5)	7.92,d(8.5)
5''	7.13,dd(1.8,8.6)	7.18,dd(8.5,1.4)	7.17,dd(8.5,1.7)
7''	7.59,d(1.8)	7.61,d(1.4)	7.62,d(1.7)
N1Me	-	-	2.09,s
N4Me	2.04,s	2.09,s	2.09,s

[a] 360 MHz (see ref. 4)

[b] 400 MHz (δ in ppm from internal TMS, multiplicity (J in Hz))

Dragmacidon B (**9**), isolated as a pale yellow powder, gave a parent ion cluster at m/z 500.0219/ 502.0196/ 504.0171 Da in the EIHRMS corresponding to a molecular formula of $C_{22}H_{22}N_4Br_2$ (ΔM +0.7/ +0.3/ -0.2 mmu).

The ^1H nmr spectrum of dragmacidon B showed resonances that integrated for a total of only eleven protons, and its proton noise decoupled ^{13}C nmr spectrum contained only eleven resonances, indicating that the molecule contained a twofold axis of symmetry. The resonances in the aromatic region of the ^1H nmr spectrum of **9** (Table 2) could be readily assigned to 6-bromoindol-3-yl residues by comparison with the data obtained for dragmacidon A (**8**). Double resonance and nOe experiments confirmed the aromatic ^1H nmr assignments. The resonances in the aliphatic regions of the ^1H (Table 2) and ^{13}C (see experimental) nmr spectra of **9** were assigned to a N,N-dimethyl-2,5-dialkyl piperazine ring by comparison with the data for dragmacidon A (**8**). It was apparent from the magnitude of the coupling constants observed for the H2 and H5 methine protons in **11** (dd, J=10.6,2.9) that the piperazine ring was in a chair conformation and that the two alkyl substituents were in equatorial orientations. Attachment of the indole rings to C2 and C5 of the piperazine ring gave the proposed structure **9** for hexindolin C.

Metabolites **7**, **8** and **9** are the first of the bis(indole) alkaloids isolated from sponges to have identical 6-bromo substitution on both indole residues. The isolation of **7**, **8** and **9** from *Hexadella* sp. represents the first time that bis(indole) alkaloids with ketoimidazole and piperazine spacers have been found in the same sponge. Dragmacidon A (**8**) showed *in vitro* cytotoxicity in the L1210 assay (ED_{50} 10 mg/mL), however, topsentin C (**7**) and dragmacidon B (**9**) were both inactive.[7]

Our discovery of metabolites **7**, **8** and **9** in SCUBA collected specimens adds further complexity to the observed variation with depth of metabolite content in *Hexadella* sp.. We now know that submersible collected specimens (-100 to -200m) contain exclusively bromotopsentin (**2**),[6] while SCUBA collected specimens (-40m) contain the two dibromotyrosine derived metabolites, hexadellins A and B,[6] as well as the three new bis(indole) alkaloids **7**, **8** and **9**. *Hexadella* sp. belongs to the order Verongida.[8] Sponges in this order typically contain metabolites derived from brominated tyrosine precursors.[9] Thus, the presence of the hexadellins in *Hexadella* sp. is consistent with the chemical taxonomy of the Verongida. The isolation of bis(indole) alkaloids from *Hexadella* is, on the other hand, without precedent among the Verongida. Therefore, the actual origin of compounds **7** to **9** remains unclear, although it seems most likely that they are not true metabolites of *Hexadella* sp.. A microbial source would be consistent with the low concentration of the bis(indole) alkaloids in *Hexadella* sp. and with the isolation of nearly identical metabolites from several other deep water sponges that belong to different poriferan orders.

Experimental

Nmr spectra were recorded on Varian XL 300 and Bruker WH 400 spectrometers. Tetramethylsilane was used as an internal standard. Low resolution mass spectra were recorded on an AEI MS 902 spectrometer and high resolution mass spectra were recorded on an AEI MS 50 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1600 Fourier Transform spectrometer.

Collection of *Hexadella* sp.: Specimens of *Hexadella* were collected by SCUBA (-40m) in Agamemnon Channel, Jervis Inlet, B.C.. Freshly collected sponge was immediately immersed in methanol. The pure bis(indole) alkaloids were obtained from the methanol extract according to the procedure described in the text.

Topsentin C (7): pale yellow powder (4 X 10^{-4} % wet wt.); IR (film) 3413, 2919, 2849, 1643, 1590 cm^{-1}; ^1H nmr (see Table 1); ^{13}C nmr (75MHz, acetone-d_6) δ 32.8, 53.5, 54.0, 115.0, 115.1, 115.5, 115.8, 116.2, 121.2, 123.3, 123.7, 124.4, 125.3, 125.4, 125.5, 126.1, 129.8, 132.0, 133.8, 157.8, 158.0; LREIMS m/z(relative intensity) 498/500/502 (38/76/38), 496/498/500 (4/8/4), 237/239 (100/100), 221/223 (30/30), 208/210 (15/15), 195/197 (20/20); HREIMS M^+ m/z 497.9672/ 499.9674/ 501.9644 ($C_{21}H_{15}N_4OBr_2$: ΔM -2.0/ +0.2/ -0.8 mmu).

Dragmacidon A (8): pale yellow glass (8 X 10^{-4}% wet wt.); IR (film) 3413, 3284, 2947, 2837, 2790, 1615, 1546cm^{-1}; ^{1}H nmr (see Table 2); ^{13}C nmr (75 MHz , acetone-d_6) δ 44.2(q), 54.1(d), 54.3(t), 63.3(d), 64.2(t), 115.0(d), 115.0(d), 115.1(s), 115.2(s), 117.5(s), 118.8(s), 122.1(d), 122.4(d), 122.4(d), 122.6(d), 123.9(d), 124.9(s), 125.0(d), 126.6(s), 138.7(s), 138.8(s); LREIMS m/z (rel. intensity) 486/488/490 (1/2/1), 291/293 (11/11), 251/252 (80/80), 221/223 (60/60), 208/210 (12/12), 195/197 (100/100); HREIMS M^{+} m/z 486.0042/ 488.0027/ 490.0027 ($C_{21}H_{20}N_4Br_2$: ΔM -1.4/ -0.9/ +0.8 mmu); nOe (**proton irradiated**: nOes observed) (**4.43 H2**: 3.29, H6a; 3.18,H3e; 7.39, H2'; 7.81, H4'), (**3.41, H5**: 2.38, H3a; 3.07, H6e; 7.35, H2"; 7.91, H4"), (**2.09, N4Me**: 3.18, H3e; 2.38, H3a; 3.41, H5;7.35, H2"; 7.91, H4").

Dragmacidon B (9): pale yellow powder (8 X 10^{-4}% wet wt.); IR (film) 3257, 2947, 2800, 1615, 1546 cm^{-1}; ^{1}H nmr (see Table 2); ^{13}C nmr (75MHz, acetone-d_6) δ 43.8(q), 63.0(d), 64.2(t), 115.2(d), 115.4(s), 117.1(s), 122.6(d), 122.8(d), 125.2(d), 126.7(s), 138.9(s); LREIMS m/z (rel. intensity) 500/502/504 (12/21/12), 422/424/ (5/5), 250/252 (7/7), 237/239 (100/100), 221/223 (60/60), 195/197 (6,6); HREIMS M^{+} m/z 500.0219/ 502.0196/ 504.0171 ($C_{22}H_{22}N_4Br_2$: ΔM +0.7/ +0.3/ -0.2 mmu).

Acetyldragmacidon A (10): 2mg of dragmacidon A (**8**) was stirred overnight at room temperature in a 1:1 mixture of freshly distilled acetic anhydride (0.5mL) and pyridine (0.5mL). The reagents were removed in vacuo and the residue purified by radial tlc to give pure **10**: ^{1}H nmr (300 MHz, $CDCl_3$) δ 1.88(s,3H), 2.15 (s,3H), 2.98 (dd, J= 1.8, 12.3Hz,1H), 3.06(dd, J=4.6, 12.3Hz,1H), 3.64 (m,1H), 3.82(dd,J=3.7,13.1Hz,1H), 4.33(bs,1H), 6.21(bs,1H), 7.20(d,J=2.6Hz,1H), 7.22(dd,J=1.7,8.6Hz,1H), 7.26(dd, J=1.7, 8.6Hz,1H), 7.49(d,J=8.5Hz,1H), 7.52(d, J=1.4Hz,1H), 7.59(d,J=1.6Hz,1H), 7.67(bs,1H0, 7.75(d,J=8.5Hz,1H), 8.20(bs,1H), 8.57(bs,1H); LREIMS M^{+} m/z 528/530/532.

Acknowledgements

Financial support was provided by grants to RJA from NSERC and the National Cancer Institute of Canada. SAM was supported by a NSERC Postgraduate Fellowship. The authors wish to thank Mike LeBlanc for assisting the collection of *Hexadella* sp. and Bill Austin for identifying the sponge.

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