New Bis(Indole) Alkaloids of the Topsentin Class from the Sponge *Spongosorites genitrix*

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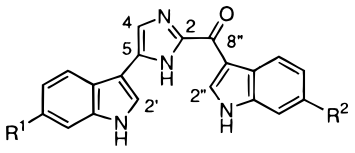
Four bis(indole) alkaloids of the topsentin class, including two new brominated compounds (**1** and **2**), have been isolated from the sponge *Spongosorites genitrix* collected from Jaeju Island, Korea. The structures of the novel compounds were determined by spectroscopic methods. These compounds exhibited moderate cytotoxicity against a human leukemia cell-line (K-562).

Bis(indole) alkaloids are widely recognized as one of the rapidly growing groups of sponge metabolites. Since topsentin, a bis(indolyl)imidazole, and its analogues were isolated from *Topsentia genitrix* (= *Spongosorites genitrix*) about a decade ago, numerous compounds of this structural class have been encountered in sponges.[1−9] Sponge-derived bis(indole) alkaloids, in particular the topsentins and their structurally related nortopsentins, exhibit potent and diverse bioactivities including antiviral, antitumor, and antiinflammatory activities.[3,7,8,10−12] The structural conciseness and wide-range bioactivity of bis(indole) alkaloids have made them attractive targets for both biomedical and synthetic purposes.[11−15]

As part of our continuing search for bioactive substances from marine organisms of Korean origin, we have collected the sponge *Spongosorites genitrix* Schmidt (family Halichondriidae) off the coast of Jaeju Island, Korea. The crude extract of the specimens exhibited potent toxicity against brine-shrimp larvae (LC_{50} 21 ppm). Guided by the results of brine-shrimp lethality and ^{1}H NMR analysis, C_{18} reversed-phase flash chromatography of the combined CH_2Cl_2 and MeOH extracts followed by silica and reversed-phase HPLC yielded four bis(indole) alkaloids of the topsentin class. Herein we report the structure elucidation and bioactivity of bromodeoxytopsentin (**1**) and isobromodeoxytopsentin (**2**), two bromodeoxy derivatives of topsentin. In addition, the specimens yielded two known metabolites, deoxytopsentin (**3**) and bromotopsentin (**4**).[16] Compounds **1** and **2** exhibited moderate cytotoxicity against a human leukemia cell-line.

As reported previously, both ^{1}H and ^{13}C NMR spectra for these metabolites in several solvents (deuterated DMSO, Me_2CO, THF, and MeOH) were very complicated due to the tautomerism (55:45 between two forms of the imidazole ring), and this severely hindered spectral interpretation.[2,3] Consequently, all of the NMR measurements were performed in 1% solutions of trifluoroacetic acid in deuterated DMSO. In this manner, the structures of two known metabolites, deoxytopsentin and bromotopsentin, were determined by a combination of spectroscopic analysis and comparison with reported data for these compounds.

Compound **1** was isolated as a yellow amorphous solid that analyzed for $C_{20}H_{13}BrN_4O$ by HREIMS. The ^{13}C NMR data for this compound were very similar to those obtained for deoxytopsentin, with the replacement of the signal of a methine carbon by that of a quaternary carbon as the only noticeable difference (Table 1). Corresponding changes were also observed in the ^{1}H NMR spectrum, in which the absence of a signal for an aromatic proton and subsequent changes of coupling patterns among the signals of aromatic protons were clearly observed.



1 R^1 = Br, R^2 = H

2 R^1 = H, R^2 = Br

3 R^1 = R^2 = H

4 R^1 = Br, R^2 = OH

The structure of **1** was determined by a combination of 2D NMR experiments. The ^{1}H COSY and HSQC data revealed the presence of a 3,5- or 3,6-disubstituted indole moiety. The distinction between these two possible structures was achieved by a NOESY experiment in which the NH proton signal at δ 11.81 (1H, d, J = 2.8 Hz) showed a strong correlation with the proton signal at δ 7.71 (1H, d, J = 1.4 Hz). The small proton−proton coupling of the latter with a signal at δ 7.29 (1H, dd, J = 8.2, 1.4 Hz), which, in turn, coupled with another signal at δ 7.88 (1H, d, J = 8.2 Hz), clearly showed that **1** possessed a 3,6-disubstituted indole moiety. This interpretation was ascertained by the HMBC data in which a three-bond correlation between signals of the H-4′ proton at δ 7.88 and the C-3′ carbon at δ 103.6 was observed. Similarly, the presence of a 3-substituted indole and an imidazole moiety was also determined by combined ^{1}H COSY, HSQC, and HMBC experiments (Table 1).

Because the HMBC experiments, performed under conditions optimized for 6 and 8 Hz of H−C coupling constants, did not exhibit long-range H−C correlations among the partial structures, the connectivity among these was established by mass analysis. The fragments at *m*/*z* 289 (rel int 40) and 287 (35), corresponding to $C_{12}H_6BrN_3O$, revealed the connection between the 6-bromoindole and the imidazole moiety, while the fragments at *m*/*z* 144 (52) and 116 (12) showed the presence of a 3-carbonylindole group

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Table 1. ^{1}H and ^{13}C NMR Assignments for Compounds **1** and **2**[a]

	1			**2**		
Carbon no.	H	C	HMBC[b]	H	C	HMBC[b]
2		141.7			142.9	
4	8.01, s	116.9	C-2, C-5	7.91, s	118.7	C-2, C-5
5		131.2			133.1	
1′	11.81, d (2.8)		C-3′, C-3a′	11.54, d (2.6)		C-2′, C-3′, C-3a′
2′	8.06, d (2.8)	127.0	C-3′, C-3a′, C-7a′	8.03, d (2.6)	124.8	C-3′, C-3a′, C-7a′
3′		103.6			105.0	
3a′		123.8			124.6	
4′	7.88, d (8.2)	121.6	C-3′, C-3a′, C-6′ C-7a′	7.98, br d (7.8)	119.7	C-6′, C-7a′
5′	7.29, dd (8.2, 1.4)	123.9	C-3a′, C-7′	7.16, br dd (7.8, 7.8)	120.3	C-3a′, C-7′
6′		115.7		7.20, br dd (8.2, 7.8)	122.3	C-4′, C-7a′
7′	7.71, d (1.4)	115.5	C-3a′, C-6′	7.48, br d (8.2)	112.3	C-3a′, C-5′
7a′		137.9			136.6	
1″	12.58, d (2.3)			12.44, d (3.4)		C-3″, C-3a″
2″	8.57, d (2.3)	139.1	C-3″, C-3a″, C-7a″	8.95, d (3.4)	138.6	C-3″, C-3a″, C-7a″
3″		114.2			113.8	
3a″		126.3			125.5	
4″	8.23, dd (7.8, 1.2)	121.9	C-3a″, C-6″, C-7a″	8.24, d (8.6)	123.3	C-5″, C-6″, C-7a″
5″	7.31, m	123.7	C-3a″	7.42, dd (8.6, 1.9)	125.4	C-3a″, C-7″
6″	7.33, m	124.8	C-4″		116.2	
7″	7.58, dd (7.8, 1.4)	113.4	C-3a″, C-5″, C-7a″	7.78, d (1.9)	115.5	C-3a″, C-5″, C-7″
7a″		137.5			137.6	
8″		172.7			174.1	

[a] ^{1}H and ^{13}C NMR data were obtained in DMSO-d_6 + TFA (ca 1%) solutions at 500 and 125 MHz, respectively. [b] Parameters were optimized for 6 and 8 Hz of H−C coupling constants. Assignments were aided by combined ^{1}H COSY, TOCSY, DEPT, HSQC, and HMBC experiments.

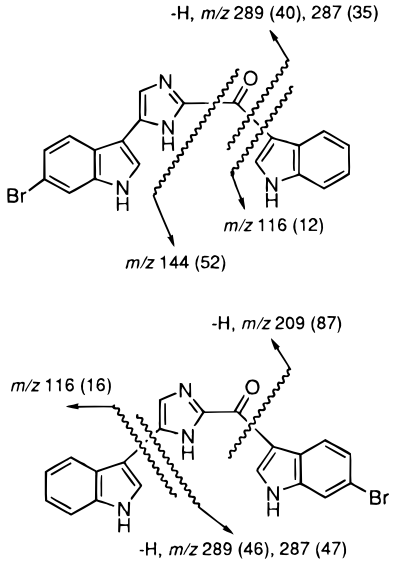

Figure 1.

(Figure 1). Thus, the structure of compound **1** was unambiguously determined as bromodeoxytopsentin (= 6′-bromotopsentin A).[16]

A closely related metabolite (**2**) was isolated as a yellow amorphous solid. The spectroscopic properties of this compound, which have the same molecular formula ($C_{20}H_{13}BrN_4O$) as **1**, were very similar to those of **1**. The combined NMR analysis, including 2D experiments, also revealed the presence of a bromoindole, an indole, and an imidazole moiety, as determined for **1**. However, careful examination of the ^{1}H NMR and ^{1}H COSY data showed that chemical shifts of several protons of **2** were significantly different from those of **1**, suggesting that the bromine was located on the indole ring of the right half of structure **2**, rather than on the left. This interpretation was confirmed by a NOESY experiment in which a correlation between the signal of a NH proton at δ 12.44 (1H, d, J = 3.4 Hz, H-1″) and that of a proton at δ 7.78 (1H, d, J = 1.9 Hz, H-7″) was observed. The small coupling constant of the latter with the signal of a proton at δ 7.42 also revealed the attachment of a bromine atom at C-6″. The NMR interpretation of **2** was supported by mass analysis (Figure 1). A major fragment at *m*/*z* 209 (rel int 87), not observed for **1**, is characteristic of the cleavage of the C−C bond between the bromoindole and the remaining part of the molecule. This fragmentation was confirmed by a high-resolution measurement. Thus, the structure of **2** was defined as isobromodeoxytopsentin (= 6″-bromotopsentin A).[16]

Topsentins and nortopsentins exhibit diverse and potent bioactivities such as cytotoxic, antitumor, antiviral, antifungal, and antiinflammatory activities as well as displacement of ligand binding to human adrenergic receptors.[3,7,8,10−12] In our measurement of bioactivity, these compounds exhibited moderate cytotoxicity against the human leukemia cell-line K-562 (LC_{50} 0.6 and 2.1 μg/mL, for **1** and **2**, respectively).

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher−Johns apparatus and are reported uncorrected. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in MeOH using a Milton−Roy spectrophotometer. NMR spectra were recorded in DMSO-d_6 solutions with 1% of trifluoroacetic acid on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si. Mass spectra were obtained by using a JEOL JMS−SX 102A mass spectrometer and provided by Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The specimens of *S. genitrix* (sample number 92J-2) were collected by hand using scuba at 20−30 m depth in November 1992, off the shore of Jaeju Island, Korea. A voucher specimen is on deposit in the sponge collection, Natural History Museum, Han Nam University, Taejeon, Korea, under the curatorship of C. J. Sim.

Extraction and Isolation. The freshly collected samples were immediately frozen and kept at −25 °C until investigated

chemically. The sponge (1.3 kg, wet wt) was lyophilized, macerated, and extracted with MeOH (3 L × 3) and CH_2Cl_2 (3 L × 2). The combined crude extracts (89.2 g) were partitioned between *n*-BuOH and H_2O. The butanol layer (56.9 g) was concentrated in vacuo, and the residue was repartitioned between *n*-hexane (8.7 g) and 15% aqueous MeOH (46.8 g). An aliquot (8.7 g) of the latter phase, which was toxic to brine-shrimp, was subjected to C_{18} reversed-phase vacuum flash chromatography using mixtures of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH), and finally EtOAc. The fraction (1.64 g) eluted with 20% aqueous MeOH was dried and separated by silica semipreparative HPLC (YMC silica column, 1 cm × 25 cm, *n*-hexane−EtOAc−THF, 5:3:2) to yield, in order of elution, compounds **1**, **3**, and **4**. Final purification was made by reversed-phase HPLC (YMC ODS-A column, MeOH−H_2O−THF, 6.5:2.5:1) to afford 239.9, 204.6, and 155.6 mg of **1**, **3**, and **4** as yellow amorphous solids, respectively.

The fraction (1.17 g) eluted with 10% aqueous MeOH from the vacuum flash chromatography was dried and separated by silica HPLC (*n*-hexane−EtOAc−THF, 6:2:2) to yield, in order of elution, compounds **1**, **3**, and **2.** Further purification was made by reversed-phase HPLC (MeOH−H_2O−THF, 6.5: 2.5:1) to yield 186.7, 79.1, and 150.3 mg of pure deoxytopsentins **1**, **2**, and **3** as yellow amorphous solids, respectively.

Bromodeoxytopsentin (1): mp 240−243 °C; IR (KBr) ν_{max} 3300 (br), 2920, 2850, 1705, 1590, 1520, 1455, 1420, 1360, 1235, 1110 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 367 (4.17), 250 (sh, 4.36), 235 (4.40), 209 (4.61) nm; ^1H and ^{13}C NMR, see Table 1; HREIMS $[M]^+$ *m/z* 406.0250 and 404.0253 (calcd for $C_{20}H_{13}{}^{81}BrN_4O$ and $C_{20}H_{13}{}^{79}BrN_4O$, 406.0255 and 404.0273, respectively); LRMS *m/z* (rel int) 406 (96), 404 (100), 289 (40), 287 (35), 144 (52), 116 (12).

Isobromodeoxytopsentin (2): mp 225−230 °C; IR (KBr) ν_{max} 3300, 2920, 2850, 1705, 1595, 1515, 1455, 1420, 1360, 1235, 1105 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 375 (4.02), 280 (4.06), 252 (4.04), 213 (4.41) nm; ^1H and ^{13}C NMR, see Table 1; HREIMS $[M]^+$ *m/z* 406.0244 and 404.0266 (calcd for $C_{20}H_{13}{}^{81}BrN_4O$ and $C_{20}H_{13}{}^{79}BrN_4O$, 406.0255 and 404.0273, respectively), $[M - C_8H_6BrN]^+$ *m/z* 209.0601 (calcd for $C_{12}H_7N_3O$, 209.0590); LRMS *m/z* (rel int) 406 (99), 404 (100), 326 (37), 289 (46), 287 (47), 210 (10), 209 (87), 163 (18), 116 (16).

Deoxytopsentin (3): mp > 280 °C (lit. > 250 °C)[3]; HREIMS $[M]^+$ *m/z* 326.1176 (calcd for $C_{20}H_{14}N_4O$, 326.1168).

Bromotopsentin (4): mp > 280 °C (lit. 296−297 °C)[3]; HREIMS $[M]^+$ *m/z* 422.0193 and 420.0217 (calcd for $C_{20}H_{13}{}^{81}BrN_4O_2$ and $C_{20}H_{13}{}^{79}BrN_4O_2$, 422.0204 and 420.0222, respectively).

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References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259−302, and references therein.
(2) Bartik, K.; Braekman, J. C.; Daloze, D.; Stoller, C.; Huysecom, J.; Vandevyver, G.; Ottinger, R. *Can. J. Chem.* **1987**, *65*, 2118−2121.
(3) Tsujii, S.; Rinehart, K. L. Jr.; Gunasekera, S. P.; Kashman, Y.; Cross, S. S.; Lui, M. S.; Pomponi, S. A.; Diaz, M. C. *J. Org. Chem.* **1988**, *53*, 5446−5453.
(4) Braekman, J. C.; Daloze, D.; Moussiaux, B.; Stoller, C.; Deneubourg, F. *Pure Appl. Chem.* **1989**, *61*, 509−512.
(5) Morris, S. A.; Andersen, R. J. *Can. J. Chem.* **1989**, *67*, 677−681.
(6) Morris, S. A.; Andersen, R. J. *Tetrahedron* **1990**, *46*, 715−720.
(7) Sakemi, S.; Sun, H. H. *J. Org. Chem.* **1991**, *56*, 4304−4307.
(8) Murray, L. M.; Lim, T. K.; Hooper, J. N. A.; Capon, R. J. *Aust. J. Chem.* **1995**, *48*, 2053−2058.
(9) Mancini, I.; Guella, G.; Debitus, C.; Waikedre, J.; Pietra, F. *Helv. Chim. Acta* **1996**, *79*, 2075−2082.
(10) Phife, D. W.; Ramos, R. A.; Feng, M.; King, I.; Gunasekera, S. P.; Wright, A.; Patel, M.; Pachter, J. A.; Coval, S. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2103−2106.
(11) McConnell, O. J.; Saucy, G.; Jacobs, R. U.S. Patent 5290777; *Chem. Abstr.* **1994**, *120*, 236178.
(12) McConnell, O. J.; Saucy, G.; Jacobs, R.; Gunasekera, S. P. U.S. Patent 5464835; *Chem. Abstr.* **1996**, *124*, 76517.
(13) Braekman, J. C.; Daloze, D.; Stoller, C. *Bull. Soc. Chim. Belg.* **1987**, *96*, 809−812.
(14) Kawasaki, I.; Yamasjita, M.; Ohta, S. *J. Chem. Soc. Chem. Commun.* **1994**, 2085−2086.
(15) Kawasaki, I.; Yamashita, M.; Ohta, S. *Chem. Pharm. Bull.* **1996**, *44*, 1831−1839.
(16) The nomenclature of these compounds varies among the earlier reports. In this paper, we follow the naming system used by Tsujii et al.[3] and Murray et al.[8]

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