(1 H, ddd, J = 7.9, 7.0, 1.2 Hz, H-5″), 3.95 (3 H, s, 1′-NMe), 3.86 (3 H, s, 1-NMe), 3.84 (3 H, s, 1″-NMe), 3.72 (3 H, s, 3-NMe), and 3.68 (3 H, s, $MeSO_4^-$); ^{13}C NMR ($CDCl_3$) δ 141.5 (s), 137.9 (s), 136.9 (s), 136.4 (d), 131.1 (d), 130.1 (s), 126.6 (s), 125.3 (d), 124.7 (s), 122.9 (d), 121.1 (d), 120.3 (d), 119.7 (d), 118.8 (d), 116.9 (s), 114.1 (d), 110.1 (d), 99.1 (s), 94.5 (s), 54.3 (q, $MeSO_4^-$), 36.3 (q), 34.8 (q), 33.8 (q), and 33.2 (q).

Biological Activities of 1–7. IC_{50} against P388 (μg/mL): 1, 7.6; 2, 7.8; 3, 1.7; 4, 2.0; 5, 7.0; 6, 0.90; 7, 0.34. MIC against *C. albicans* (μg/mL): 1, 3.1; 2, 6.2; 3, 12.5; 4, >50; 5, >50; 6, >50; 7, >50.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds 1–3, 6, and 7 (10 pages). Ordering information is given on any current masthead page.

Notes

A Brominated (Aminoimidazolinyl)indole from the Sponge *Discodermia polydiscus*

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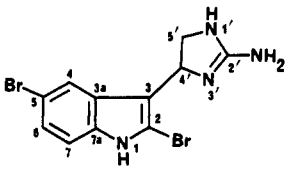
A wide variety of bioactive imidazole alkaloids derived from aromatic amino acids were reported from marine invertebrates.[1] Representative structures included aplysinopsins,[2] topsentins,[3] polyandrocarpamide D,[4] and naamidines.[5] During the course of our search for antitumor compounds from marine organisms, we isolated a novel brominated (aminoimidazolinyl)indole, designated as discodermindole (1), from the sponge *Discodermia polydiscus* DuBocage 1879 (family Theonellidae, order Lithistida).[6] In in vitro assays, 1 yielded IC_{50} values of 1.8 μg/mL against P388 (murine leukemia), 4.6 μg/mL against A-549 (human lung), and 12 μg/mL against HT-29 (human colon) cell lines. Its isolation and structure elucidation are reported herein.



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Samples of *Discodermia polydiscus* were collected by Johnson-Sea-Link submersible at a depth of 185 m off Chub Cay, Barry Islands, Bahamas, in August 1985. Freshly collected sponge specimens were immediately frozen and extracted later with methanol to give an extract that was active in our antitumor screening panels. The extract was partitioned between ethyl acetate and water. The aqueous fraction was lyophilized and triturated with 1:1 chloroform–methanol. Centrifugal countercurrent chromatography of the resulting oily extract, followed by Sephadex LH-20 chromatography and HPLC, yielded an active component, discodermindole (1).

Table I. ^1H (360 MHz) and ^{13}C (90 MHz) NMR Data of Discodermindole (1)[a]

atom	$\delta(^1H)$ (m, J, Hz)	$\delta(^{13}C)$ (m[b])	long-range coupled ^1H[c]
2		111.7 (s)	H4′
3		111.8 (s)	H4, H5′
3a		126.7 (s)	H4, H7
4	7.57 (d, 1.8)	119.8 (d)	H6
5		112.3 (s)	H4, H6, H7
6	7.24 (dd, 8.6, 1.8)	124.3 (d)	H4
7	7.32 (d, 8.6)	113.5 (d)	
7a		135.5 (s)	H4, H6, H7
2′		159.8 (s)	H4′, H5′
4′	5.23 (dd, 10.1, 7.1)	51.2 (d)	H5′
5′	3.99 (dd, 10.1, 10.1)	48.5 (t)	
	3.53 (dd, 10.1, 7.1)		

[a] Recorded in DMSO-d_6. [b] Multiplicity deduced from DEPT. [c] Observed from COLOC, HETCOR, and HETCOSY.

The molecular formula of 1 was determined to be $C_{11}H_{10}Br_2H_4$ by high resolution FAB mass spectrometry. EIMS failed to show the molecular ion but exhibited a 1:2:1 molecular ion cluster at m/z 273/275/277 corresponding to $C_8H_5Br_2N$, indicative of a dibromoindole moiety. The UV spectrum showed absorptions at 224 (ϵ 34 000), 282 (5700), 292 (6100) and 300 nm (5000), characteristic of an indole chromophore.[7] The coupling pattern of three aromatic ^1H NMR signals at δ 7.24 (dd, J = 8.6 and 1.8

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(1) Faulkner, D. J. *Nat. Prod. Rep.* **1990**, *7*, 269 and references cited therein.

(2) (a) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, 61. (b) Hollenbeak, K. H.; Schmitz, F. J. *Lloydia* **1977**, *40*, 479. (c) Tymiak, A. A.; Rinehart, K. L.; Bakus, G. J. *Tetrahedron* **1985**, *41*, 1039.

(3) (a) Batrik, K.; Braekman, J.; Daloze, D.; Stoller, C.; Huysecom, J.; Vandevyver, G.; Ottinger, R. *Can. J. Chem.* **1987**, *65*, 2118. (b) Tsujii, S.; Rinehart, K.; Gunasekera, S.; Kashman, Y.; Cross, S.; Lui, M.; Pomponi, S.; Diaz, M. *J. Org. Chem.* **1988**, *53*, 5446. (c) Morris, S.; Anderson, R. *Tetrahedron* **1990**, *46*, 715.

(4) Lindquist, N.; Fenical, W. *Tetrahedron Lett.* **1990**, *31*, 2521.

(5) (a) Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193. (b) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003. (c) Ciminiello, P.; Fattorusso, E.; Magno, S.; Mangoni, A. *Tetrahedron* **1989**, *45*, 3873.

(6) Van Soest, R. W. M.; Stentoft, N. *Studies Fauna Curacao Caribbean Islands* **1988**, *70*, 1.

(7) Scott, A. I. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: Oxford, 1964; pp 172–178.

0022-3263/91/1956-4307$02.50/0

Hz), 7.32 (d, J = 8.6 Hz), and 7.57 (d, J = 1.8 Hz), along with the occurrence of three protonated and five nonprotonated sp^2 carbons in the ^{13}C NMR spectra, indicated the existence of a 2,3,5- or 2,3,6-trisubstituted indole ring. Two ^{13}C NMR signals at 126.7 (s) and 135.5 (s) were typical of C-3a and C-7a, respectively, of the indoles.[3,8,9] Further ^1H and ^{13}C NMR assignments for the indole ring, based on consideration of coupling information from COLOC,[10] HETCOR,[11] and HETCOSY[12] experiments (Table I), enabled us to conclude that 1 contained the 2,5-dibromoindol-3-yl moiety.

The remaining subunit as required by the molecular formula was $C_3H_6N_3$. The presence of a guanidine functionality was suggested from the positive Sakaguchi test[13] and a ^{13}C NMR signal at δ 159.8 (s).[14] Two other carbons including a methylene at δ 48.5 (t) and a methine at 51.2 (d), observed in the ^{13}C NMR and DEPT spectra, were nitrogen-bearing from chemical shift arguments. ^1H NMR and COSY spectra illustrated that the corresponding methylene protons (H-5′) at δ 3.99 (dd, J = 10.1, 10.1 Hz) and δ 3.53 (dd, J = 10.1, 7.1 Hz) were geminally coupled, and both protons were coupled to the methine proton (H-4′) at δ 5.23 (dd, J = 10.1, 7.1 Hz). Further 2D NMR experiments (Table I) showed that the guanidino carbon (C-2′) was long-range coupled with H-5′ and H-4′. These data established the presence of the 2-amino-2-imidazolin-4-yl structure feature in 1. Furthermore, long-range correlations were observed from C-2 to H-4′, from C-3 to H-5′, and from C-3a to H-4′, thereby connecting the C-4′ to C-3 to yield the structure 3-(2-amino-2-imidazolin-4-yl)-2,5-dibromoindole[15] for 1. The stereochemistry of the chiral center at C-4′ was not assigned. It may be of chemotaxonomic interest that discodermindole is structurally unrelated to the discodermins[16] and the calyculins,[17] reported previously from sponges of the genus *Discodermia*.

Experimental Section

Isolation of Discodermindole (1). A taxonomic voucher specimen of the sponge *D. polydiscus* was deposited at Harbor Branch Oceanographic Museum (catalog no. 003:00058). The sponge (88 g wet weight) was stored frozen and extracted with MeOH (250 mL × 3). The extract was concentrated to dryness under reduced pressure and then partitioned between EtOAc (100 mL) and H_2O (100 mL). The aqueous layer was lyophilized and triturated with $CHCl_3$–MeOH (1:1, 50 mL × 2). After evaporation of the organic solvent, the oily residue (810 mg) was fractionated by using centrifugal countercurrent chromatography ($CHCl_3$–MeOH-H_2O, 5:10:6, lower phase stationary) to give 30 fractions. Active fractions 18 and 19 were pooled and chromatographed on a Sephadex LH-20 column with MeOH, followed by HPLC on an NH_2 column with $CHCl_3$–MeOH (3:1), to yield 1 (15 mg, 0.017% of wet sponge) as a colorless viscous oil: $[\alpha]^{20}_D$ = −27° (c 1.0, MeOH); HRFABMS MH$^+$ 358.9336 (calcd for $C_{11}H_{11}{}^{79}Br^{81}BrN_4$, Δ −0.6 mmu); HREIMS M$^+$ − $C_3H_5N_3$ 272.8779 (calcd for $C_8H_5{}^{79}Br_2N$, Δ 0 mmu); LREIMS 277/275/273 (rel intensity 49/100/49), 196/194 (41/46), 115 (80), 83 (77), and 57 (59); IR (KBr) ν_{max} 3300 (br), 1670, 1570, 1410, 1330, 1090, 910, and 795 cm^{-1}; ^1H and ^{13}C NMR, Table I.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compound 1 (2 pages). Ordering information is given on any current masthead page.

(8) Katritzky, A. R. *Handbook of Heterocyclic Chemistry*; Pergamon Press: Oxford, 1985; p 61.
(9) Moaeles-Rios, M.; Espineira, J.; Joseph-Nathan, P. *Magn. Reson. Chem.* **1987**, *25*, 377.
(10) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosi, H. *J. Magn. Reson.* **1984**, *57*, 331.
(11) Bax, A.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 501.
(12) Sato, Y.; Geckle, M.; Gould, S. *Tetrahedron Lett.* **1985**, *26*, 4019.
(13) Jepson, J. B.; Smith, J. *Nature* **1953**, *172*, 1100; **1955**, *177*, 84.
(14) (a) Toda, F.; Oshima, T.; Ishida, Y.; Takehira, Y.; Saito, K.; Tanaka, K. *Handbook of ^{13}C NMR Spectra*; Sandyo Publishing: Tokyo, 1981; p 144. (b) Kobayashi, J.; Masashi, T.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Noise, S. *Tetrahedron* **1990**, *46*, 5579.
(15) The preferred IUPAC name was provided by Dr. K. L. Loening, Chemical Abstracts Service.
(16) (a) Matsunaga, S.; Fusetani, N.; Konosu, S. *Tetrahedron Lett.* **1984**, *25*, 5156; **1985**, *26*, 855. (b) Matsunaga, S.; Fusetani, N.; Konosu, S. *J. Nat. Prod.* **1985**, *48*, 236.
(17) (a) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780. (b) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K. *J. Org. Chem.* **1988**, *53*, 3930.

Evidence for Equatorial Bridging in 2,2′-Bis(hexahydropyrimidines), Perhydro-4,5,8a,9a-tetraazafluorenes, and Perhydro-3a,4a,7a,8a-tetraazacyclopentanofluorenes through One-Bond C–H Coupling

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Introduction

2,2′-Bis(hexahydropyrimidines) are molecules that in principle can reside in either of two minimum energy conformations, A or B (Chart II). A study of one tetranitro derivative, 1,1′,3,3′-tetranitro-2,2′-bis(hexahydropyrimidine) (1; Chart I), by X-ray crystallography has shown it to exist entirely in conformation B.[1] In contrast, the molecular structures of free amines 4 and 5, as determined by X-ray crystallography, are consistent with conformation A.[2] However there has been no report of the preferred arrangement of derivatives in solution.[3] This aspect was of interest to us in connection with a study of novel heterocyclic systems derived from bis(hexahydropyrimidines)[1] and was relevant when predictions were to be made of the relative configuration of the substituted heterocycles. The present paper concerns an NMR spectroscopic study of the stereochemistry of 2,2′-bis(hexahydropyrimidines) and related cyclopentano-fused tri- and tetracyclic compounds (Chart I), as their free amines, in solution.

Results and Discussion

The synthesis of compounds **2** and **3** has been described.[1] They are readily converted into tri- and tetracyclic derivatives **6–10** and **12–15**, respectively, through condensation with aldehydes and, in the case of **10**, with acetone.[1]

(1) Black, D. StC.; Craig, D. C.; Giitsidis, O.; Read, R. W.; Salek, A.; Sefton, M. A. *J. Org. Chem.* **1989**, *54*, 4771.
(2) Black, D. StC.; Craig, D. C.; Kassiou, M.; Read, R. W. *Aust. J. Chem.* **1991**, *44*, 143.
(3) One paper that describes rotamer populations of *N,N′,N″,N‴*-tetranitroso-2,2′-bis(hexahydropyrimidine) by NMR spectroscopy has been published: Willer, R. L.; Moore, D. W. *Magn. Reson. Chem.* **1988**, *26*, 95.

0022-3263/91/1956-4308$02.50/0