Ugibohlin: A New Dibromo-seco-isophakellin from *Axinella carteri*†

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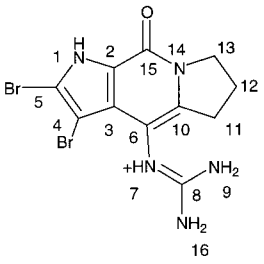
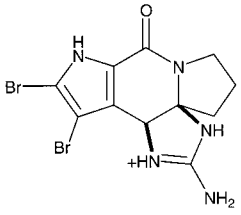
Chemical investigation of a marine sponge, *Axinella carteri*, collected on a reed slope of Talakanen Island, Phillipines, has afforded the new metabolite ugibohlin (**1**), along with its known cyclic derivative dibromoisophakellin (**2**). Structure elucidation of the isolated metabolites involved high-field 2D NMR spectroscopy including $^1H-^1H$ COSY, HSQC, and HMBC. Revised chemical shift assignments are provided for **2**.

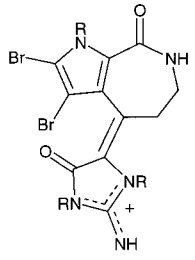
Bromopyrrole alkaloids are characteristic secondary metabolites found in marine Porifera belonging to several genera including *Axinella, Agelas, Acanthella, Pseudaxinyssa*, and *Hymeniacidon*.[1] Examples of these metabolites include hymenialdisine,[2] ageliferins,[3] oxysceptrin,[4] manzacidins,[5] konbu'acidin,[6] tauroacidins,[7] spongiacidins,[9] dibromoisophakellin,[10] and dibromocantharelline.[11] Many are reported to have intriguing biochemical activity. For instance, the hymenialdisines are potent inhibitors of kinase activity.[12] We have now chemically investigated organic extracts of a specimen of *Axinella carteri* (Dendy, 1889) (Demospongiae, Halichondrida, Axinellidae), collected from Talakanen Island, Phillipines. This afforded a new bromopyrrole alkaloid, ugibohlin (**1**), and the known dibromoisophakellin (**2**).



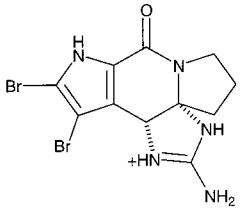
1 ugibohlin



2 dibromoisophakellin



3 R=H spongiacidin A
3a R=CH_3 trimethylspongiacidin A



2a dibromocantharelline

Ugibohlin **1** showed pseudomolecular ion peaks at *m/z* 388, 390, and 392 in a 1:2:1 ratio in its ESI mass spectrum. A molecular formula of $C_{11}H_{12}Br_2N_5O_2$ was confirmed by HRESIMS. The ^{13}C NMR spectrum exhibited 11 signals corresponding to three methylenes and eight fully substituted sp^2 carbons. ^1H and ^{13}C NMR data of **1** (Table 1) were similar to those of the known compound dibromoisophakellin (**2**)[10] and its enantiomer, dibromocantharelline (**2a**).[11] The ^{13}C NMR spectrum of **1**, however, differed from that of dibromoisophakellin (**2**) and dibromocantharelline (**2a**) by the presence of two nonprotonated sp^2 resonances at δ_C 102.8 and δ_C 142.9 ppm, rather than signals for a methine carbon at δ_C 53.9 and a quaternary carbon at δ_C 84.1. The ^1H NMR spectrum of **1** also lacked the methine signal (δ 5.21, H-6) found in the corresponding spectrum of dibromoisophakellin (**2**) and dibromocantharelline (**2a**). These differences clearly implied that **1** was the C-10−N-9 seco-derivative of dibromoisophakellin (**2**) or dibromocantharelline (**2a**) and that the C-6−C-10 bond was unsaturated. The signal at δ_C 102.6 in the ^{13}C NMR spectrum of **1** was assigned to C-6 and the signal at δ_C 142.0 to C-10 on the basis of chemical shift considerations and HMBC connectivities as indicated in Table 1.

High-resolution mass measurements and ^1H and ^{13}C NMR data for compound **2** were consistent with that for dibromoisophakellin (**2**)[10] and dibromocantharelline (**2a**).[11] Its negative optical rotation indicated that compound **2** was the former. We were, however, intrigued by differences in the chemical shifts attributed to C-4 and C-5 for dibromoisophakellin (**2**)[10] and dibromocantharelline (**2a**)[11] because, obviously, these differences could not be attributed to differences in configuration. Carbons 4 and 5 were attributed respectively in dibromoisophakellin **2** at δ_C 108.8 and 122.8 and at δ_C 122.7 and 108.7 for the dibromocantharelline (**2a**). In our case, no significant C/H long-distance correlation could be exploited to unambiguously assign C-4 and C-5 in compound **2**. HMBC correlations between H-6, N*H*-7, and N*H*-9 and a signal at δ_C 156.8 assigned this chemical shift to C-8. Similarily, an HMBC correlation was detected between H-13a and C-15, indicating a carbonyl assignment at δ_C 154.7 ppm. These data reverse published assignments made for C-8 and C-15 in dibromoisophakellin (**2**)[10] and dibromocantharelline (**2a**).[11]

An extensive literature search on dibromopyrroles was undertaken in order to detect a similarity pattern among chemical shift assignments of those well-described moieties. The most useful piece of information came from the structure elucidation of spongiacidin A (**3**), where the authors describe 2D NMR correlations of its trimethyl derivative (**3a**).[9] By analogy we attributed C2 at 122.8 ppm, C3 at 122.4 ppm, C4 at 96.3 ppm, and C5 at 108.4 ppm, revising those previously reported data for dibromoisophakellin (**2**)[10] and dibromocantharelline (**2a**).[11]

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† In memoriam of Eugene Bohl, deceased April 1998 at age 30.

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Table 1. ^{13}C, ^{1}H NMR, and HMBC Data for Compound **1**[a]

position	δ_C	δ_H (mult, *J* in Hz)	gHMBC[b]
1		13.43 (s)	
2	127.4		
3	123.7		
4	89.7		
5	113.3		
6	102.8		H-11b
7		8.88 (s)	
8	157.7		NH-7
9		not detected	
10	142.9		NH-7, H-12, H-11a, H-11b
11a	28.3	2.85 (m)	H-12
11b		3.00 (m)	
12	21.1	2.13 (2H, m)	H-11a, H-11b
13a	48.4	3.97 (m)	
13b		4.10 (m)	
15	151.7		
16		7.25 (s)	

[a] In DMSO-d_6. [b] Proton showing long-range correlation to indicated carbon.

Experimental Section

General Experimental Procedure. The IR and UV spectra were taken on Nicolet Protégé 460 and Beckman DU-600 spectrophotometers, respectively. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 at 25 °C on a Varian Inova spectrometer (11.7 T Oxford Magnet) operating at 500 and 125 MHz, respectively, using residual solvent signals as an internal reference. All 2D NMR experiments were performed on the same spectrometer and in the same solvent. ESIMS and HRESIMS were obtained on a Mariner electrospray-time-of-flight biospectrometry workstation from PerSeptive Biosystems.

Animal Material. *Axinella carteri* (NCI 1083) was supplied by Prof. D. J. Faulkner, Scripps Institute of Oceanography, La Jolla, CA. It was collected in the Phillipines, on a reed slope of Talakanen Island in March of 1992. This sponge, originally described as being of bright orange color, was identified as *Axinella carteri* (Dendy, 1889) (Demospongiae, Halichondrida, Axinellidae) by Dr. John N. A. Hooper, Director, Queensland Center for Biodiversity, Queensland Museum, Australia. It has been registered in the Queensland Museum as QM G318544.

Extraction and Isolation. A fresh freeze-dried collection of *Axinella carteri* (10 g) was suspended in excess CH_2Cl_2 overnight and the solvent discarded. The *Axinella* residue was resuspended in excess MeOH for further overnight soaking. The solvent mixture was then dried in vacuo to give a reddish-brown oil. This oil was subjected to semipreparative reversed-phase HPLC (Alltech Alltima C_{18}, 1.0 × 25.0 cm, 5 μM) using a MeCN−0.01%TFA linear gradient (10−100% over 45 min). The flow rate was 3 mL/min. Detection was at 210 nm. A fraction collected between 12 and 14 min was dried and resubjected to semipreparative reversed-phase HPLC (YMC ODS SH-434-10 S-10 120A AQ, 2.0 × 25.0 cm) using a MeCN−0.01%TFA linear gradient (1−30% over 70 min). The flow rate was 5 mL/min. Detection was at 210 nm. The fraction collected between 66 and 68 min afforded dibromoisophakellin (**2**) (4.0 mg) as a yellow-brown oil. The fraction collected between 60 and 62 min was dried, then resubjected to semipreparative reversed-phase HPLC (Alltech Alltima C_{18}, 1.0 × 25.0 cm, 5 μM). This time the elution conditions were as follows: isocratic at 1% MeCN in 0.01% TFA for 10 min followed by a linear gradient to give 30% MeCN−0.01% TFA at 70 min. The flow rate was 3 mL/min. Detection was at 210 nm. A fraction collected between 43 and 44 min afforded ugibohlin (**1**) (3.0 mg) as an amorphous white solid.

Ugibohlin (1): amorphous white solid; UV (MeOH) λ_{max} (log ϵ) 208 (3.83) 240 (4.25) 242 (4.27) 269 (3.60) nm; IR (KBr) ν_{max} 3160, 1661, 1601, 1580 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); HRESIMS *m/z* 389.9411 (calcd for $C_{11}H_{12}{}^{79}Br^{81}BrN_5O$, 389.9389).

Table 2. ^{13}C, ^{1}H NMR, COSY, and gHMBC Data for **2**[a]

position	δ_C	δ_H (mult, *J* in Hz)	COSY	gHMBC[b]
1		13.32 (s)		
2	122.8			H-6, NH-1, NH-7
3	122.4			H-6
4	96.3			H-6, NH-1, NH-7, NH-9
5	108.4			H-6, NH-1
6	53.9	5.21 (d, 1.2)	NH-7	H-11, NH-7, NH-9
7		8.84 (t, 1.4)	H-6, NH-9	
8	156.8			H-6, NH-7, NH-9
9		9.81 (d, 1.8)	NH-7	
10	84.1			H-6, H-11, H-12, H-13a, H-13b, NH-7, NH-9
11a	38.9	2.20 (m)	H-12	H-6, H-11, H-13a, H-13b
12	19.1	2.00 (m)	H-11, H-13a, H-13b	H-11, H-13a, H-13b
13a	44.1	3.45 (m)	H-12, H-13b	H-11, H-12
13b		3.55 (m)	H-12, H-13a	
15	154.7			H-13a
16		7.97 (br s)		

[a] In DMSO d_6. [b] Proton showing long-range correlation to indicated carbon.

Dibromoisophakellin (2): yellow-brown oil; $[\alpha]_D$ −53.7 (*c* 0.00053 g/mL, MeOH); ^{1}H and ^{13}C NMR (see Table 2); HRESIMS *m/z* 389.9382 (calcd for $C_{11}H_{12}{}^{79}Br^{81}BrN_5O$, 389.9389).

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References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55, and references therein.
(2) (a) Cimino, G.; De Rosa, S.; De Stefano, S.; Mazzarella, L.; Puliti, R.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 767−768. (b) Kitagawa, I.; Kobayashi, M.; Kitanaka, K.; Kido, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1983**, *31*, 2321−2328.
(3) Kobayashi, J.; Tsuda, M.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Nozoe, S. *Tetrahedron* **1990**, *46*, 5579−5586.
(4) Kobayashi, J.; Tsuda, M.; Ohizumi, Y. *Experientia* **1991**, *47*, 301−304.
(5) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574−4576.
(6) Kobayashi, J.; Suzuki, M.; Tsuda, M. *Tetrahedron* **1997**, *46*, 15681−15684.
(7) Kobayashi, J.; Inaba, K.; Tsuda, M. *Tetrahedron* **1997**, *46*, 16679−16682.
(8) Sharma, G. M.; Buyer, J. S.; Pomerantz, M. W. *J. Chem. Soc., Chem. Commun.* **1980**, 435−436.
(9) Inaba, K.: Sato, H.; Tsuda, M.; Kobayashi, J. *J. Nat. Prod.* **1998**, *61* (5), 693−695.
(10) Fedoreyev, S. A.; Utkina, N. K.; Ilyin, S. G.; Reshetnyak, M. V.; Maximov, O. B. *Tetrahedron Lett.* **1986**, *27*, 3177−3180.
(11) De Nanteuil, G.; Ahond, A.; Guilhem, J.; Poupat, E.; Tran Huu Dau, E.; Potier, P.; Pusset, M.; Pusset, J.; Laboute, P. *Tetrahedron* **1985**, *41*, 6019−6033.
(12) Meijer, L.; Thunissen; A. M. W. H.; White, A. W.; Garnier, M.; Nikolic, M., Tsai, L. H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Kim, S. H.; Pettit, G. R. *Chem. Biol.* **2000**, *7*, 51−63.

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