An Analysis of Phakellin and Oroidin Structures Stimulated by Further Study of an *Agelas* Sponge†

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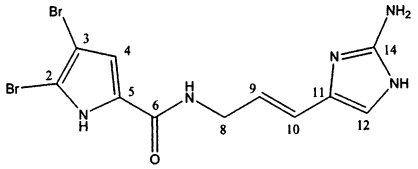
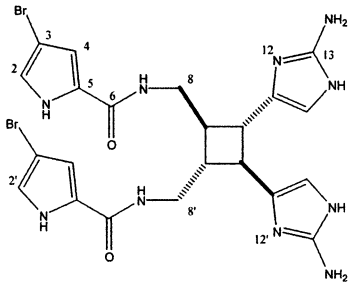
Two new phakellin alkaloids, (−)-7-*N*-methyldibromophakellin (**14**) and (−)-7-*N*-methylmonobromophakellin (**15**), were isolated from an *Agelas* sp. sponge, collected near Wewak, Papua New Guinea. Inhibition assays employing both 12- and 15-human lipoxygenase isozymes (12-HLO, 15-HLO) were used to guide the isolation of **14**, and LCMS data pointed the way to uncovering **15**. The structure elucidations were completed by spectroscopic data analysis and comparisons to the properties of known phakellins. The lipoxygenase IC_{50} data showed that **14** was modest in its selective inhibition of 12-HLO. The phakellin family is uniquely marine-derived, and comments are offered on the biogenetic insights provided by these new structures.

The oroidin class of alkaloids is defined by the signature bromopyrrole carboxamide and aminoimidazole moieties connected through a propyl chain. The phakellin family of compounds, first discovered in 1969, is now considered to be a subset of the oroidin alkaloid class. These compounds are of historic importance in marine natural products chemistry because they were among the first complex, halogenated alkaloids to be described. The elucidation of their structures presented a challenge, but these efforts were rewarded by their accompanying biological activity. Both the Scheuer and Faulkner research groups contributed to these efforts.[1,2] In 1971, oroidin (**1**) was isolated from *Agelas oroides*;[3a] however, a revised structure was soon proposed.[3b] It was not until 1981, during solid-state photodimerization studies of (−)-sceptrin (**2**) by Faulkner and Clardy,[2] that the final structure of **1** was confirmed by X-ray diffraction analysis. In 1977, the Scheuer group reported midpacamide (**3**) from an unidentified sponge[1a] and later outlined the heralded immunosuppressant (−)-palau'amine (**4**) from *Stylotella aurantium*.[1b,c]

The lead compounds of the phakellin family, namely, (−)-dibromophakellin (**5**) and (−)-monobromophakellin (**7**), along with an unidentified antibacterial agent, were first encountered from *Phakellia flabellata*[4] (often confused with *Acanthella* sp.[5a]). Also included in that report were (−)-**6**, the *N*-acetylated form of (−)-**5**, and phakellin (**8**), the catalytic hydrogenation product of both (−)-**5** and (−)-**7**. The absolute stereochemistry of (−)-**6** was assigned the 6*S*,10*R* configuration by X-ray diffraction analysis, and this information was used to assign similar 6*S*,10*R* configurations to (−)-**5**, (−)-**7**, and **8**. Likewise, this method was applied to determine the 6*R*,10*S* stereochemistry of (+)-dibromophakellin (**5′**) from *Pseudaxinyssa cantharella*[6] and derivatives (+)-**6′** and (+)-phakellin (**8′**). A summary of these assignments and their relationship with optical rotations are shown in Figure 1. Related compounds



oroidin (**1**)



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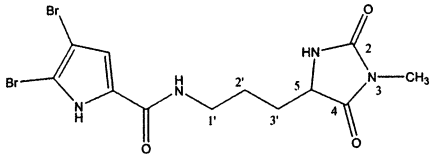
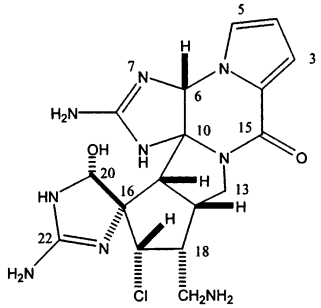


midpacamide (**3**)



(−)-palau'amine (**4**)

† Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

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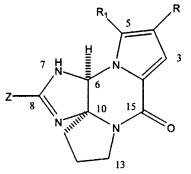
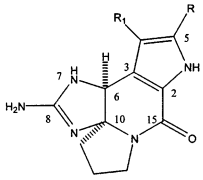


6*S*, 10*R*

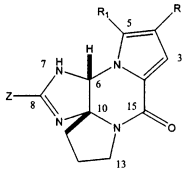
(–)-dibromophakellin (**5**) [4c]
$[\alpha]_D$ = -200° Z=NH_2; R=R_1=Br
(–)-17-*N*-acetyl of **5** (**6**) [4c]
$[\alpha]_D$ = -221° Z=NH_2; R=R_1=Br
(–)-monobromophakellin (**7**) [4c]
$[\alpha]_D$ = -123° Z=NH_2; R=Br, R_1=H
phakellin (**8**) [4c]
$[\alpha]_D$ not reported Z=NH_2; R=R_1=H
(–)-phakellstatin (**9**) [8a]
$[\alpha]_D$ = -70° Z=OH; R=R_1=Br



6*S*, 10*R*

(–)-dibromoisophakellin (**10**) [9]
$[\alpha]_D$ = -101° R=R_1=Br

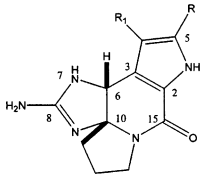
(–)-isophakellin (**12**) [9]
$[\alpha]_D$ = -27° R=R_1=H



6*R*, 10*S*

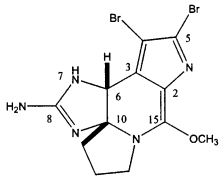
(+)-dibromophakellin (**5'**) [6]
$[\alpha]_D$ = +159° Z=NH_2; R=R_1=Br
(+)-17-*N*-acetyl of **5'** (**6'**) [6]
$[\alpha]_D$ = +121° Z=NH_2; R=R_1=Br

(+)-phakellin (**8'**) [6]
$[\alpha]_D$ = +5° Z=NH_2; R=R_1=H
(+)-phakellstatin (**9'**) [8a]
$[\alpha]_D$ = +69° Z=OH; R=R_1=Br



6*R*, 10*S*

(+)-dibromocantharelline (**10'**) [6]
$[\alpha]_D$ = +95° R=R_1=Br
(+)-17-*N*-acetyl of **10'** (**11'**) [6]
$[\alpha]_D$ = +9° R=R_1=Br
(–)-cantharelline (**12'**) [6]
$[\alpha]_D$ = -2° R=R_1=H



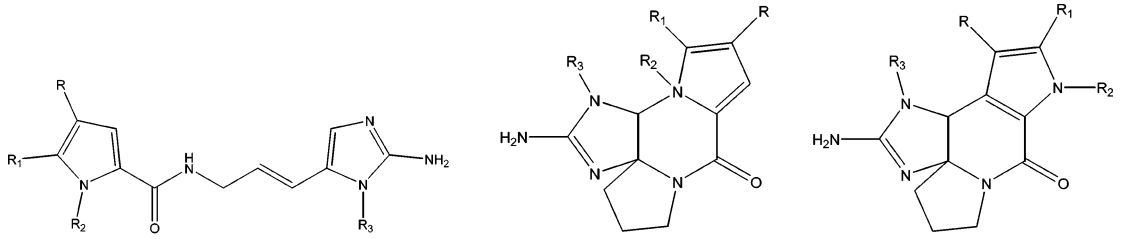
6*R*, 10*S*
(+)-*O*-methyldibromocantharelline (**13'**)[6]
$[\alpha]_D$ = +95°

Figure 1. Stereochemical variations of phakellin-related structures.

(−)-dibromophakellstatin (**9**) from *Phakellia mauritiana*[7] and its enantiomer, (+)-dibromophakellstatin (**9′**), obtained from synthesis,[8] extend this relationship. Additionally, the absolute configurations and optical rotation data of both (−)-dibromoisophakellin (**10**) from *Acanthella carteri*[9] and its debrominated form, (−)-isophakellin (**12**), follow this trend. Finally, (+)-dibromocantharelline (**10′**) and its 17-*N*-acetylated form, (+)-**11′**, were each assigned the 6*R*,10*S* configuration.[6] Interestingly, while diazomethane treatment of (+)-**10′** led to (+)-*O*-methyldibromocantharelline (**13′**), the hydrogenation of (+)-**10′** led surprisingly to (−)-cantharelline (**12′**), whose anomalous rotation suggests it was scalemic. From a structural perspective, oroidin (**1**) is related to (−)-dibromophakellin (**5**) by a complex cyclization of **1** that formally connects the pyrrole nitrogen atom (N1) to the unalkylated aminoimidazole carbon (C6) and the amino nitrogen atom (N14) to the alkylated aminoimidazole carbon (C10). A different, but equally complex cyclization, in which the latter connection remains the same, but the former is instead the pyrrole β-carbon (C2) to C6, describes the isophakellin-cantharelline skeleton.

During the past three decades, the sponges that are the sources of **1**−**13** have been intensely studied. Astoundingly, more than 75 sponge-derived oroidin alkaloids have been reported from these and other sponges. Our interest in the oroidin alkaloids began in 1994 with the discovery of the biogenetically complex mauritamide A from *Agelas mauritiana*.[10] Recently, a universal chemical pathway was proposed to explain the biogenesis leading to 15 categorized oroidin-based structural families, including the phakellins.[11] Biomimetic[12] and traditional syntheses[8b,13] of the uniquely marine phakellins offer further biosynthetic insight. Presented here are the structures of new oroidin alkaloids (−)-7-*N*-methyldibromophakellin (**14**) and (−)-7-*N*-methylmonobromophakellin (**15**) isolated from an *Agelas* sp. sponge. These compounds were obtained using a combination of bioassay- and LCMS-guided fractionation.

Table 1. Possible Structural Permutations of the Phakellin Alkaloids and Their Oroidin Congeners

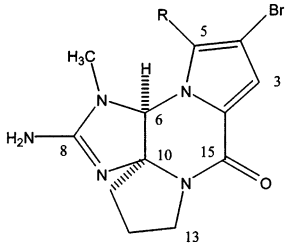


A B C

type	R	R_1	R_2	R_3	compound	source
A	H	H	H	H	clathrodin (**17**) [a]	*Agelas clathrodes*
A	Br	H	H	H	hymenidin (**16**) [b]	*Hymeniacidon* sp.
A	Br	H	CH_3	H	unknown (**22**)	
A	Br	H	H	CH_3	keramidine (*Z* alkene) (**19**) [c]	*Agelas* sp.
A	Br	Br	H	H	oroidin (**1**) [d]	*Agelas oroides*
A	Br	Br	CH_3	H	sventrin (**18**) [e]	*Agelas sventres*
A	Br	Br	H	CH_3	unknown (**23**)	
B	H	H	H	H	phakellin (**8**) [a]	hydrogenation
B	Br	H		H	(−)-monobromophakellin (**7**) [f]	*Phakellia flabellata*
B	Br	H	CH_3	H	unknown and unlikely (**27**)	
B	Br	H		CH_3	(−)-7-*N*-methylmonobromophakellin (**15**)	*Agelas* sp.
B	Br	Br		H	(−)-dibromophakellin (**5**) [f]	*Phakellia flabellata*
B	Br	Br	CH_3	H	unknown and unlikely (**28**)	
B	Br	Br		CH_3	(−)-7-*N*-methyldibromophakellin (**14**)	*Agelas* sp.
C	H	H	H	H	(−)-cantharelline (**12′**) [g]	hydrogenation
C	Br	H	H	H	monobromoisophakellin (**20**) [h]	*Agelas* sp.
C	Br	H	CH_3	H	unknown (**24**)	
C	Br	H	H	CH_3	unknown (**25**)	
C	Br	Br	H	H	(−)-dibromoisophakellin (**10**) [i]	*Acanthella carteri*
C	Br	Br	CH_3	H	(−)-*N*-methyldibromoisophakellin (**21**) [j]	*Stylissa caribica*
C	Br	Br	H	CH_3	unknown (**26**)	

[a] Morales, J. J.; Rodriguez, A. D. *J. Nat. Prod.* **1991**, *54*, 629−631. [b] Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. *Experientia* **1986**, *42*, 1176−1177. [c] Nakamura, H.; Ohizumi, Y.; Kobayashi, J. *Tetrahedron Lett.* **1984**, *25*, 2475−2478. [d] (a) isolation: Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *Chem. Commun.* **1971**, 1129−1130. (b) revised structure elucidation: Garcia, E. E.; Benjamin, L. L.; Fryer, R. I. *J. Chem. Soc., Chem. Commun.* **1973**, 78−79. (c) X-ray analysis: Walker, R. P.; Faulkner, D. J.; Van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 6772−6773. [e] Assmann, M.; Zea, S.; Köck, M. *J. Nat. Prod.* **2001**, *64*, 1593−1595. [f] (a) Burkholder, P. R.; Sharma, G. *Lloydia* **1969**, *32*, 466−483. (b) Sharma, G.; Burkholder, P. R. *Chem. Commun.* **1971**, 151−152. (c) Sharma, G.; Magdoff-Fairchild, B. *J. Org. Chem.* **1977**, *42*, 4118−4124. [g] De Nanteuil, G.; Ahond, A.; Guilhem, J.; Poupat, C.; Tran Huu Dau, E.; Potier, P.; Pusset, M.; Pusset, J.; Laboute, P. *Tetrahedron* **1985**, *41*, 6019−6033. [h] Assmann, M.; Köck, M. *Z. Naturforsch.* **2002**, *57*, 153−156. [i] Fedoreyev, S. A.; Utkina, N. K.; Ilyin, S. G.; Reshetnyak, M. V.; Maximov, O. B. *Tetrahedron Lett.* **1986**, *27*, 3177−3180. [j] Assmann, M.; van Soest, R. W. M.; Köck, M. *J. Nat. Prod.* **2001**, *64*, 1345−1347.

Their structures were determined via spectroscopic and comparative data analyses.



6*S*, 10*R*

(–)-7-*N*-methyldibromophakellin (**14**) R=Br $[\alpha]_D$ = -20°
(–)-7-*N*-methylmonobromophakellin (**15**) R=H $[\alpha]_D$ = -35°

Results and Discussion

The initial hurdle during our reinvestigation of the phakellin subset of oroidin alkaloids was to gain command of the spectroscopic handles to differentiate among the previously known structures. Three general structural types can be defined as illustrated in Table 1 and are based on the two-ring-containing framework type A (e.g., **1**) versus the tetracyclic systems, which exist in two regio forms, type B (e.g., **5**) and type C (e.g., **10**). The base structural formula of $C_{11}H_{13}N_5O$ is isomeric for all types, which can be further characterized by their patterns of bromination and nitrogen methylation. The entire known phakellin family and their related oroidins, including the two new metabolites we isolated, can be described by the general structures A−C. The relationships in Table 1 assisted dereplication efforts by providing a template of structural motifs to which mass spectroscopy and NMR data were compared. Furthermore, in addition to the known structures in Table 1, predicted structures **22**−**28**, which have not been isolated to date, represent undiscovered alkaloids of this class.

The previously undescribed *Agelas* sp. (UCSC coll. no. 98108) was collected near Wewak, Papua New Guinea, in 1998, preserved, and transported back to UCSC laboratories. Our Kupchan-like extraction method was followed, and the sponge dichloromethane extract was subjected to Sephadex LH-20 column chromatography eluted with methanol. The resulting eight fractions were subjected to LCMS analysis and screened for 12-HLO and 15-HLO inhibition as previously described.[14] Three consecutively eluting fractions exhibited promising 12-HLO selectivity, which increased proportionally to a broadly eluting compound showing a dibrominated isotopic molecular ion

Table 2. ^{13}C (125.7 MHz), ^{1}H (500 MHz), and DEPT NMR Spectral Data for **14** in DMSO-d_6 and ^{1}H (500 MHz) NMR Spectral Data for **15** in MeOH-d_4

	14			**15**
no.	δ_C	δ_H	DEPT	δ_H
2	125.5			
3	116.3	7.05 (s)	CH	7.12 (d)
4	102.5			
5	108.0			6.90 (d)
6	72.6	6.24 (s)	CH	5.95 (s)
8	158.4			
10	82.4			
11	36.0	2.16 (m)	CH_2	2.55 (m)
12	18.9	2.10 (m)	CH_2	2.41 (m)
		1.98 (m)		2.19 (m)
13	42.9	3.68 (m)	CH_2	3.99 (m)
		3.43 (m)		3.81 (m)
15	153.7			
17	33.0	3.01 (s)	CH_3	2.94 (s)

cluster of 402, 404, 406 $[M + H]^+$. Using semiprepatory reversed-phase HPLC, compound **14** was isolated. Closer analysis of LCMS data of the parent dichloromethane sponge extract showed another compound with an isotopic molecular ion cluster of 324, 326 $[M + H]^+$, suggesting the monobrominated form of **14**. Prepatory reversed-phase HPLC of this extract then afforded **15**. The structural characterization of these compounds relied heavily on mass spectrometry information and signature NMR peaks.

Accurate mass data of **14** was provided by ESITOF and established the molecular formula (401.9546 $[M + H]^+$, calcd for $C_{12}H_{14}N_5OBr_2$, 401.9559). Dereplication began by using this formula as a search seed,[15] and four possible compounds were revealed, (+)-*O*-methyldibromocantharelline (**13′**),[6] sventrin (**18**),[16] keramidine (**19**),[17] and (−)-*N*-methyldibromoisophakellin (**21**).[18] However, structures **13′**, **18**, **19**, and **21** were eliminated as possibilities by comparing their ^{13}C and ^{1}H NMR properties to those of **14**, whose data are shown in Table 2. First, **14** displayed an amide carbonyl (δ_C 153.7), a guanidinium carbon (δ_C 158.4), and a downfield quaternary carbon (δ_C 82.4) characteristic of the phakellin family. Next, a side-by-side comparison of **14** with **5** showed that all ^{13}C shifts were nearly identical in DMSO-d_6, but an additional shift (δ_C 33.0, δ_H 3.01) in **14** made it clear that the *N*-methyl congener of **5** was in hand. This was confirmed by gHMBC correlations (see Figure 1), which further demonstrated the methyl position to be at N7 and not N9. Interestingly, this agrees with the results of Sharma,[4b] who analyzed the tautomeric forms of the aminoimidazole ring of **5**, **7**, and **8** and suggested the double bond was at the C8−N9 position. Furthermore, while shift assignments for the brominated pyrrole vary in the phakellin literature, our gHMBC data confirmed our ^{13}C shift assignments and agreed with a previous study of polybrominated pyrroles functionalized at C2 with α-carbonyl groups.[19]

Assigning stereochemistry of (−)-**14** began by comparing its optical rotation with those of the known phakellins. The trend shown in Figure 1 suggested that (−)-**14** possessed stereochemistry similar to that of (−)-**5**, (−)-**6**, and (−)-**7**; however, the large difference in $[\alpha]_D$ values among this series prompted further investigation. Data were obtained from NOESY NMR experiments (see Figure 3), and correlations between H6 and H11 clearly revealed their *cis* relationship. Thus, the absolute stereochemistry of (−)-**14** was assigned as 6*S*,10*R*.

The isolation of (−)-7-*N*-methylmonobromophakellin (**15**) was not bioassay guided, but rather took advantage of LCMS screening data. Following the characterization of **14**,

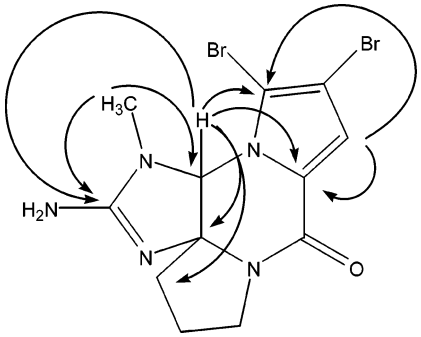


Figure 2. $^{1}H-^{13}C$ gHMBC NMR correlations for (−)-7-*N*-methyldibromophakellin (**14**) in DMSO-d_6.

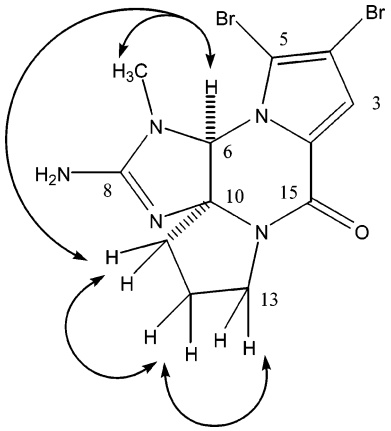


Figure 3. $^{1}H-^{1}H$ NOESY NMR correlations for (−)-7-*N*-methyldibromophakellin (**14**) in DMSO-d_6.

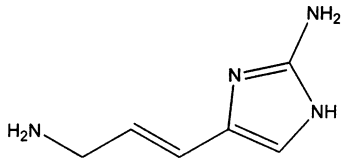
and keeping in mind the phakellin structural permutations, the LCMS data of the parent dichloromethane extract was examined for compounds with monobrominated isotopic molecular ion clusters. Only one component in the complex mixture was found, and it showed a low-resolution peak of 324, 326 $[M + H]^+$. Following the isolation of **15** via reversed-phase HPLC, accurate mass data established the molecular formula (324.0453 $[M + H]^+$, calcd for $C_{12}H_{14}N_5OBr$, 324.0454). Structure elucidation was determined by ^{1}H NMR data comparisons with **14** (see Table 2). Comparison of optical rotation between that of (−)-**15** and (−)-**14** suggested the same stereochemistry for this pair, and thus, (−)-**15** is also assigned the 6*S*,10*R* configuration.

Oroidin alkaloids have demonstrated broad bioactivity, the most celebrated being the immunosuppressant properties of palau'amine (**4**) from *S. aurantium*.[3] None, however, have been examined for human lipoxygenase inhibition. Although **14** was not defined as potent, it was moderately selective toward 12-HLO, with the following IC_{50} values: 12-HLO $IC_{50} = 10.7 \pm 1.3\ \mu M$; 15-HLO $IC_{50} = 48 \pm 16\ \mu M$. The 12-HLO isozyme has been implicated in psoriasis[20] and cancer cell proliferation[21] via its role in the production of leukotrienes and lipoxins. Compound **15** was not tested due to small sample amount.

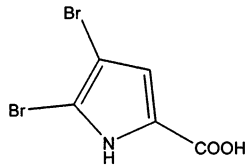
Conclusions

The novel compounds **14** and **15** from *Agelas* sp. add some missing links to the family of phakellins. From the extensive reports of the these alkaloids, it is clear that they occur reliably, and most often, from the genus *Agelas*, regardless of geographic location; however, other sponges have also yielded phakellins, prompting chemotaxonomic studies.[22] Furthermore, although no feeding studies have been performed, the recently proposed biogenetic pathway to the phakellins[11] was based on biogenetic precursors

3-amino-1-(2-aminoimidazoleyl)prop-1-ene (**29**)[23] and 4,5-bromopyrrole-2-carboxylic acid (**30**).[24] The tautomerization/cyclization reactive pathways proposed, as well as evidence from biomimetic[12] and traditional syntheses[13] of phakellins, support the structural relationships presented in Table 1. Known compounds **1**, **5**, **7**−**8**, **10**, **12**, and **14**−**21** differ only in their patterns of carbon−nitrogen skeleton cyclization, bromination, and/or nitrogen methylation. It is reasonable, therefore, to expect that the congeners **22**−**26** may already exist in nature and/or are reasonable synthetic targets. The comprehensive family of phakellin permutations shown provides a knowledge base for these metabolites through structural relationships. Biological activity and SAR investigations of the entire phakellin family should prove useful.



3-amino-1-(2-aminoimidazoleyl)prop-1-ene (**29**)



4,5-bromopyrrole-2-carboxylic acid (**30**)

Experimental Section

General Experimental Procedures. The NMR spectra were recorded at 500 MHz for ^{1}H NMR and 125.7 MHz for ^{13}C NMR. Multiplicities of ^{13}C NMR were determined from DEPT and gHMBC (500 MHz). Accurate mass spectral data were obtained using electrospray ionization, time-of-flight detection (ESITOFMS). Chromatography was performed using Sephadex LH-20 (gel permeation) and ODS (reversed-phase HPLC).

Collection and Identification. The *Agelas* sp. sponge specimen was collected using scuba from two proximal sites near the coast of Wewak, Papua New Guinea (coll. no. 98108), approximately 50 feet deep. Matching some taxonomic markers for the genus *Agelas* (order Agelasida; family Agelasidae),[5b] the specimen was massive ramose with deformed branches of varied diameter, regular oscules (1−3 mm di.), compressible, but rubbery, and brown to dark pink externally and tan internally. However, the sample was identified as an undescribed *Agelas* sp. (order Agelasida; family Agelasidae) by Dr. M. C. Diaz (UCSC), who noted particular spicule features including large verticillated acanthostyles (250−130 × 12−20 μm), usually bent once, and similar size verticillated oxeas. The fresh sponges were preserved, transported to our lab, and extracted as previously described.[10] An underwater photograph and a voucher sample are available from P.C. (UCSC).

Extraction and Isolation. The sponge material was first extracted with methanol, then with methylene chloride. The methanol extract was initially partitioned between water and methylene chloride. The aqueous layer was extracted with *sec*-butanol (WB), and the *sec*-butanol dried to give a fraction (WB). The organic layer was dried and partitioned between 9:1 MeOH/H_2O and hexane, and the hexane was dried to give a fraction (FH). The aqueous layer was then adjusted to 1:1 MeOH/H_2O and partitioned against CH_2Cl_2, producing two fractions (FM and FD, respectively). To complete the process of extraction, the methylene chloride extract from the sponge was partitioned between 9:1 MeOH/H_2O and hexane, to give two fractions (DMM and DMH, respectively).

The 98108 DMM partition (500 mg) was subjected to Sephadex LH-20 chromatography eluted with 100% methanol to yield eight fractions. Fraction 3 (S3) was purified by reversed-phase HPLC to produce (−)-7-*N*-methyldibromophakellin (**14**) (3.5 mg), which eluted as a broad peak due to the high basicity of the guanidinium group.[4c,25] The 98108 DMM partition was also separated using prepatory reversed-phase HPLC to afford (−)-7-*N*-methylmonobromophakellin (**15**) (1.2 mg), also eluting broadly.

Lipoxygenase Assay. Enzymatic activity of purified (95%) 12-HLO and 15-HLO human reticulocyte enzymes was determined using previously described procedures.[14a] These involve direct measurement of the product formation following the increased absorbance at 234 nm (25 mM HEPES, pH 8, ∼3 μM arachadonic acid for 12-HLO, and 25 mM HEPES, pH 7.5, ∼3 μM linoleic acid for 15-HLO). All reactions were performed in 2 mL of buffer and ∼200 nM enzyme and constantly stirred with a rotating bar (∼22 °C). The inhibitors were typically dissolved in methanol at a concentration of ∼1 mg/mL. IC_{50} values were determined by measuring the enzymatic rate at a variety of inhibitor concentrations (depending on the inhibitor potency) and plotting their values versus inhibitor concentration. The corresponding data were fit to a simple saturation curve, and the inhibitor concentration of 50% activity was determined (IC_{50}). We define potent compounds as those with IC_{50} values < 1 μM and selective compounds as those exhibiting IC_{50} ratios of either 10-fold greater or smaller than unity.

(−)-7-*N*-Methyldibromophakellin (14): white powder; $[\alpha]^{28}_D$ −19.8° (*c*, 1.4 mg/mL MeOH); UV λ_{max} (CH_3OH) 238 (ϵ 6780), 280 (5430); see Table 2 for ^{1}H and ^{13}C NMR; HRESITOFMS $[M + H]^+$ *m*/*z* 401.9546 (calcd for $C_{12}H_{14}N_5OBr_2$ 401.9559).

(−)-7-*N*-Methylmonobromophakellin (15): white powder; $[\alpha]_D^{28}$ −35.0° (*c*, 1.0 mg/mL MeOH); UV λ_{max} (CH_3OH) 238 (ϵ 1700), 280 (1280); see Table 2 for ^{1}H and ^{13}C NMR; HRESITOFMS $[M + H]^+$ *m*/*z* 324.0453 (calcd for $C_{12}H_{14}N_5OBr$ 324.0454).

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