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N-Methylated Ageliferins from the Sponge *Astrosclera willeyana* from Pohnpei

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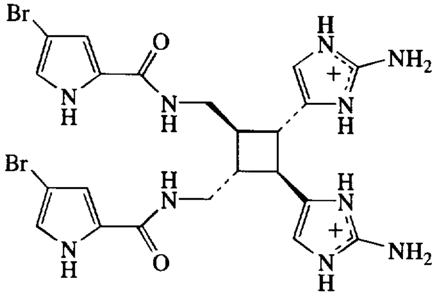
Abstract: The sponge *Astrosclera willeyana* contains seven new bromopyrrole alkaloids (**3-9**), together with the known alkaloids bromoageliferin (**10**) and dibromoageliferin (**11**). The structures of the new alkaloids (**3-9**), ageliferins that are methylated on one or both of the pyrrole nitrogens, were elucidated by interpretation of spectral data. Chemotaxonomic relationships involving bromopyrrole alkaloids are discussed in the light of this research.

Bromopyrrole alkaloids of the "oroidin" series are considered to be one class of metabolites that are characteristic of sponges of the genus *Agelas*, the other classes being terpenoids of the "agelasine" and "agelasidine" series.[1] There are however reports that some Axinellidae[2] and Ceratoporellida[3] contain pyrrole-2-carboxylic acid derivatives.[1] Dimers of oroidin, such as sceptrin (**1**) from *Agelas sceptrum*[4] and ageliferin (**2**) from *A. conifera*, *A.* cf. *mauritiana*,[2,5] and an *Agelas* species from Okinawa[6] represent interesting bioactive structural variations. In this paper, we report the isolation of seven new *N*-methylated ageliferins (**3**)-(**9**) together with the known metabolites bromoageliferin (**10**) and dibromoageliferin (**11**) from a calcareous sponge *Astroscera willeyana* from Pohnpei.



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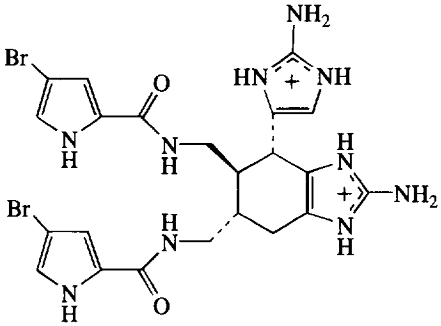
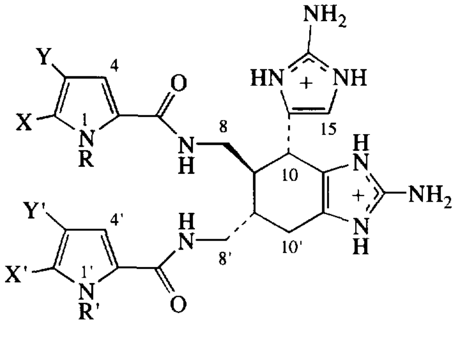


2

A methanol-chloroform (1:1) extract of *Astroscera willeyana* was partitioned to obtain an aqueous methanol-soluble fraction that was separated by chromatography on Sephadex LH-20, using methanol as eluant, followed by reversed phase HPLC to obtain *N*(1')-methylageliferin (**3**, 0.24% dry wt.), *N*(1),*N*(1')-dimethylageliferin (**4**, 0.16% dry wt.), *N*(1')-methylisoageliferin (**5**, 0.36% dry wt.), *N*(1),*N*(1')-



3 R = X = X' = H, R' = Me, Y = Y' = Br
4 R = R' = Me, X = X' = H, Y = Y' = Br
5 R = X = Y' = H, R' = Me, X' = Y = Br
6 R = R' = Me, X = Y' = H, X' = Y = Br
7 R = X' = H, R' = Me, X = Y = Y' = Br
8 R = X = H, R' = Me, X' = Y = Y' = Br
9 R = H, R' = Me, X' = X = Y = Y' = Br
10 R = R' = X' = H, X = Y = Y' = Br
11 R = R' = H, X = X' = Y = Y' = Br

dimethylisoageliferin (**6**, 0.18% dry wt.), *N*(1')-methyl-2-bromoageliferin (**7**, 0.30% dry wt.), *N*(1')-methyl-2'-bromoageliferin (**8**, 0.28% dry wt.), and *N*(1')-methyl-2,2'-dibromoageliferin (**9**, 0.06% dry wt.), together with the known metabolites bromoageliferin (**10**, 0.08% dry wt.) and dibromoageliferin (**11**, 0.12% dry wt.), that were identified by comparison of their spectral data with literature values.[5,6]

N(1')-Methylageliferin (**3**), obtained as a trifluoroacetate salt, is a pale yellow glass with no sharp melting point. The molecular formula, $C_{23}H_{26}Br_2N_{10}O_2$, was inferred from a high resolution mass measurement of the protonated molecular ion. Comparison of the ^1H and ^{13}C NMR spectra with those of ageliferin (**2**) led to the conclusion that **3** was an *N*-methyl derivative of ageliferin. The ^1H and ^{13}C NMR spectra were assigned (see Tables 1 and 2, respectively) by analysis of the COSY, HMQC, and HMBC experiments but this still left several critical issues to be addressed; the location of the *N*-methyl group, the bromine substitution pattern, the regiochemistry of pyrrolecarboxamide substitution on the cyclohexene ring and the relative stereochemistry about the cyclohexene ring.

The location of the *N*-methyl group on one of the two pyrrole rings was determined by observation of HMBC correlations between the methyl signal at δ_H 3.95 and carbon signals at δ 127.0 and 129.0, which are characteristic of C-2/C-5 in a pyrrole ring. The ^1H and ^{13}C NMR signals for the 3-bromo-*N*-methylpyrrole 5-carboxamide and 3-bromopyrrole 5-carboxamide ring systems were assigned as shown in Tables 1 and 2 by using the combination of HMQC, HMBC, and COSY data. The NMR data for the

Table 1. ^{1}H NMR data [500 MHz, MeOH-d_4, (mult, *J* in Hz)] for *N*(1′)-methylageliferin (**3**), *N*(1),*N*(1′)-dimethylageliferin (**4**), *N*(1′)-methylisoageliferin (**5**), *N*(1),*N*(1′)-dimethylisoageliferin (**6**), *N*(1′)-methyl-2-bromoageliferin (**7**), *N*(1′)-methyl-2′-bromoageliferin (**8**), *N*(1′)-methyl-2,2′-dibromoageliferin (**9**), and bromoageliferin (**10**).

H#	**3**	**4**	**5**	**6**
2	6.93 (d, 1.5)	6.90 (d, 1.5)	6.95 (d, 1.5)	6.91 (d, 1.5)
2′	6.88 (d, 1.5)	6.90 (d, 1.5)		
3′			6.20 (d, 4)	6.21 (d, 4)
4	7.26 (d, 1.5)	7.10 (d, 1.5)	7.25 (d, 1.5)	7.14 (d, 1.5)
4′	6.81 (d, 1.5)	6.86 (d, 1.5)	6.87 (d, 4)	6.86 (d, 4)
8	3.97 (br d, 14)	3.97 (dd, 15, 3)	3.94 (dd, 14, 4)	3.93 (br d, 14)
8	3.40 (dd, 14, 4)	3.39 (dd, 15, 5)	3.47 (dd, 14, 4)	3.43 (dd, 14, 4)
8′	3.86 (br d, 14)	3.89 (br d, 14)	3.84 (br d, 14)	3.86 (dd, 15, 3)
8′	2.98 (dd, 14, 10)	3.05 (dd, 14, 9)	3.07 (dd, 14, 10)	3.06 (dd, 14, 9)
9	1.98 (m)	2.03 (m)	2.05 (m)	2.04 (m)
9′	2.16 (m)	2.17 (m)	2.19 (m)	2.19 (m)
10	3.73 (br d, 8)	3.77 (br d, 8)	3.76 (br d, 8)	3.76 (br d, 8)
$10'_{eq}$	2.82 (dd, 16, 4)	2.81 (dd, 16, 5)	2.83 (dd, 16, 4)	2.83 (dd, 16, 5)
$10'_{ax}$	2.42 (m, 16, 9, 3)	2.44 (m, 16, 9, 3)	2.45 (m, 16, 9, 3)	2.44 (m, 16, 9, 3)
15	6.84 (br s)	6.84 (br s)	6.84 (br s)	6.84 (br s)
NMe		3.92 (s, 3H)		3.91 (s, 3H)
N'Me	3.95 (s, 3H)	3.95 (s, 3H)	3.97 (s, 3H)	3.95 (s, 3H)

H#	**7**	**8**	**9**	**10**
2	6.95 (d, 1.5)			
2′	6.91 (d, 1.5)			6.95 (d, 1.5)
4	7.30 (s)	7.23 (d, 1.5)	6.96 (s)	7.02 (br s)
4′	6.81 (d, 1.5)	6.96 (s)	6.90 (s)	6.85 (br s)
8	3.96 (br d, 14)	3.95 (br d, 14)	3.76 (dd, 14, 4)	3.74 (dd, 15, 5)
8	3.45 (dd, 14, 5)	3.45 (dd, 14, 4)	3.38 (m)	3.51 (dd, 15, 5)
8′	3.86 (br d, 14)	3.85 (br d, 14)	3.70 (br d, 14)	3.64 (dd, 15, 3)
8′	3.00 (dd, 14, 10)	3.03 (dd, 14, 10)	3.35 (dd, 14, 9)	3.30 (dd, 14, 9)
9	1.98 (m)	2.01 (m)	2.17 (m)	2.17 (m)
9′	2.16 (m)	2.17 (m)	2.23 (m)	2.27 (m)
10	3.73 (br d, 8)	3.74 (br d, 8)	3.83 (br d, 8)	3.83 (br d, 8)
$10'_{eq}$	2.82 (dd, 16, 5)	2.82 (dd, 16, 4)	2.71 (dd, 16, 5)	2.77 (dd, 16, 5)
$10'_{ax}$	2.42 (m, 16, 9, 3)	2.43 (m, 16, 9, 3)	2.46 (m, 16, 9, 3)	2.48 (m, 16, 8, 2)
15	6.86 (br s)	6.84 (br s)	6.79 (br s)	6.81 (br s)
NMe				
N'Me	3.96 (s, 3H)	4.01 (s, 3H)	3.96 (s, 3H)	

3-bromopyrrole-5-carboxamide ring agreed well with literature values.[5,6] Comparison of ^{1}H and ^{13}C NMR data, including the magnitude of the coupling constants revealed that the substitution pattern and stereochemistry about the cyclohexene ring in **3** were the same as for **10** and **11**, and analysis of the COSY experiment allowed the H_2-8 and H_2-8′ methylene protons to be unambiguously assigned. The lack of HMBC correlations across the amide bonds initially prevented the location of the two different pyrrole rings but molecular modeling revealed the possibility of nOe correlations between the amide -NH signals and the adjacent H-4 signals. The ^{1}H NMR spectrum was recorded in DMSO-d_6 and the chemical shifts of the NH-7 and NH-7′ signals at δ 8.03 and 8.36, respectively, were assigned by analysis of a COSY spectrum: irradiation of these signals cause negative 16-17% enhancements[7] of the H-4 and H-4′ signals at δ 7.08 (dd, 1H, J = 3, 1.5 Hz) and 6.89 (d, 1H, J = 1.5 Hz), respectively (Table 3), which were assigned on the basis of the additional H-4/NH-1 coupling in the non-methylated ring. These data firmly established the regiochemistry and relative stereochemistry of *N*(1′)-methylageliferin (**3**).

Table 2. ^{13}C NMR Data (50 MHz, MeOH-d_4) for ageliferins **3-10** (* adjacent signals may be interchanged).

C#	3	4	5	6	7	8	9	10
2	123.0	129.0*	123.0	129.1	106.4	123.1	106.4	106.6
2′	129.0	129.1*	111.1	111.2	129.1	112.8	112.9	123.1
3	97.5	95.4	97.6	95.5	100.0	97.6	100.0	100.3
3′	95.5	95.4	111.5	111.5	95.5	99.1	99.1	97.6
4	114.3	116.3*	114.3	116.4	115.2	114.2	114.6	115.2
4′	115.8	115.8*	114.8	114.7	115.8	116.0	116.2	113.8
5	127.2	127.0	127.1	127.1	128.4	127.1	128.6	127.6
5′	127.0	127.0	127.9	128.0	127.0	128.5	128.6	127.3
6	163.3	163.5*	163.3	163.9	162.5	163.3	163.2	163.0
6′	163.6	163.8*	163.9	163.9	163.6	163.0	162.1	162.5
8	38.7	39.1	39.0	39.0	38.8	38.9	40.6	40.1
8′	43.0	42.8	42.9	43.0	43.0	43.1	42.5	42.8
9	44.1	43.9	44.1	44.2	44.2	44.1	43.5	44.1
9′	37.5	37.6	37.5	37.6	37.5	37.4	37.3	37.1
10	33.3	33.7	33.4	33.5	33.4	33.3	34.1	33.2
10′	25.0	25.0	24.8	24.9	25.0	24.9	23.9	23.7
11	127.0	127.0	127.3	127.5	127.0	127.1	127.4	128.4
11′	123.0	122.9	123.0	123.0	123.0	123.0	122.8	123.0
13	149.2*	149.3*	149.0*	149.0*	149.2*	149.3*	149.0*	149.1
13′	149.1*	149.1*	149.1*	149.0*	149.1*	149.2*	149.4*	149.1
15	113.4	113.3	113.3	113.3	113.5	113.4	113.1	113.1
15′	119.5	119.6	119.4	119.6	119.5	119.5	119.4	119.1
NMe		37.4*		37.2				
N′Me	37.7	37.7*	35.3	35.4	37.7	36.7	36.4	

Table 3. Nuclear Overhauser effect difference spectroscopy (NOEDS) data.

Compound	H-irr	H-obs	% enhancement	H-irr	H-obs	% enhancement
3	NH-7	H-4	-16	NH-7′	H-4′	-17
5	NH-7	H-4	-19	NH-7′	H-4′	-18
6	NH-7	H-4	-13	NH-7′	H-4′	-12
7	NH-7	H-4	-17	NH-7′	H-4′	-17
8	NH-7	H-4	-18	NH-7′	H-4′	-15
9	NH-7	H-4	-12	NH-7′	H-4′	-27

N(1),*N*(1′)-Dimethylageliferin (**4**), obtained as a hydrochloride salt, is a pale butter-milk colored glass. The molecular formula, $C_{24}H_{28}Br_2N_{10}O_2$, suggested that **4** was a homologue of **3** and, indeed, an additional *N*-methyl signal was observed in the ^1H NMR spectrum (δ_{NMe} = 3.92, 3.95). In the ^{13}C NMR spectrum, the signals due to the two pyrrole rings were almost coincidental with the signals for C-2′ to C-5′ in **3**, indicating that pyrrole rings were brominated at C-3 and C-3′. The relative stereochemistry of *N*(1),*N*(1′)-dimethylageliferin (**4**), which is identical to that of **3**, was established by comparison of the ^1H and ^{13}C NMR data of **3** and **4**.

N(1′)-Methylisoageliferin (**5**) was obtained as the TFA salt, which is a pale yellow glass. The molecular formula, $C_{23}H_{26}Br_2N_{10}O_2$, indicated that **5** is an isomer of alkaloid **3**. The upfield regions of both the ^1H and ^{13}C NMR spectra were nearly identical for both compounds but changes in the downfield regions indicated a different bromination pattern in one of the pyrrole rings. In the ^1H NMR spectrum there were two pairs of pyrrole protons at δ 6.95 (d, 1H, J = 1.5 Hz) and 7.25 (d, 1H, J = 1.5 Hz) and at 6.20 (d, 1H, J = 4 Hz) and 6.87 (d, 1H, J = 4 Hz): the latter pair of signals can be attributed to a 2-bromopyrrole ring. A complete assignment of the NMR data was accomplished using HMQC and HMBC experiments. In the HMBC experiment, there were correlations from the *N*-methyl signal at δ_H 3.97 to quaternary carbon signals at δ 111.1 (C-2′) and 127.9 (C-5′), indicating the presence of an N-methyl-2-bromopyrrole ring. Once again, the positions of the two pyrrole rings relative to the cyclohexene ring were determined by nOe measurements recorded in DMSO-d_6 (Table 3).

N(1),*N*(1′)-Dimethylisoageliferin (**6**), obtained as a hydrochloride salt, is an off-white glass. The molecular formula, $C_{24}H_{28}Br_2N_{10}O_2$, together with the similarity of the ^1H NMR spectra, which differ mainly by the presence of an additional *N*-methyl signal (δ_{NMe} = 3.92, 3.95), suggested that **6** was a homologue of **5**. Comparison of the ^{13}C NMR spectra revealed a characteristic 6 ppm downfield shift of the C-2 signal from δ 123.0 to 129.1 on *N*-methylation of the pyrrole ring. The same 6 ppm downfield shift for the C-2 signal is observed in going from *N*(1′)-methylageliferin (**3**) to *N*(1),*N*(1′)-dimethylageliferin (**4**). A lesser but consistent 2 ppm downfield shift was observed for the C-4 signal. A complete analysis of the HMQC and HMBC data confirmed the assignments given in Tables 1 and 2. The regiochemistry of the pyrrole rings was again determined by performing NOEDS experiments on a DMSO-

d_6 solution of **6** (Table 3), resulting in the identification of *N*(1),*N*(1′)-dimethylisoageliferin (**6**).

N(1′)-Methyl-2-bromoageliferin (**7**) and *N*(1′)-methyl-2′-bromoageliferin (**8**), both isolated as TFA salts, were obtained as off-white glasses. Both compounds had the same molecular formula, $C_{23}H_{25}Br_3N_{10}O_2$, having one more bromine than the previous compounds. Examination of the ^{1}H NMR spectra revealed that the upfield regions were again almost identical to that of other ageliferins. Since **7** and **8** are isomeric, it seemed logical to propose that the additional bromine atom was on one or other of the pyrrole rings, but this did not exclude the possibility of regioisomers with regard to position of the methylated pyrrole rings. In the ^{1}H NMR spectrum of **7**, the pyrrole proton signals were at δ 6.81 (d, 1 H, J = 1.5 Hz), 6.91 (d, 1 H, J = 1.5 Hz), and 7.30 (s, 1H), confirming that the extra bromine was on one of the pyrrole rings. In the ^{13}C NMR spectrum, the signals due to the N-methyl pyrrole ring exactly matched those of the *N*-methyl-3-bromopyrrole ring in **3**, leading to the conclusion that the additional bromine was on the non-methylated pyrrole ring. Comparison of the ^{13}C NMR signals assigned to the dibromopyrrole ring in **7** with data for bromoageliferin (**10**) and dibromoageliferin (**11**) provided convincing evidence for the 2,3-dibromo substitution pattern. In the ^{1}H NMR spectrum of **8**, the pyrrole proton signals were at δ 6.95 (d, 1 H, J = 1.5 Hz), 6.95 (s, 1H), and 7.23 (d, 1 H, J = 1.5 Hz); comparison of these data with the spectra of **3** and **5** reveals that the doublets are characteristic of a 3-bromopyrrole-5-carboxamide ring. This assignment was confirmed by comparing the corresponding ^{13}C NMR data for **3**, **5**, and **8**. The additional bromine atom must therefore be on the *N*-methylated pyrrole ring and comparison of the ^{13}C NMR data for the *N*-methyl-2,3-dibromopyrrole ring with that of the 2,3-dibromopyrrole ring revealed the same downfield shift of 6 ppm for C-2 that had been observed above. The regiochemistry of pyrrole substitutions on the cyclohexene rings of **7** and **8** were again established by NOEDS experiments (Table 3).

The TFA salt of *N*(1′)-methyl-2,2′-dibromoageliferin (**9**) was isolated as an off-white glass. The molecular formula, $C_{23}H_{24}Br_4N_{10}O_2$, together with the presence of two pyrrole proton signals at δ 6.90 (s, 1H) and 6.96 (s, 1H) and an N-methyl signal at 3.96 (s, 3H) in the ^{1}H NMR spectrum, suggested that **9** was simply an N-methyl derivative of dibromoageliferin (**11**). Comparison of the ^{13}C NMR chemical shifts, assigned by interpretation of HMQC and HMBC experiments, for the pyrrole carbons in **9** with the comparable data for **7** and **8** confirmed the presence of one 2,3-dibromopyrrole ring and one N-methyl-2,3-dibromopyrrole ring in **9**. The regiochemistry of the pyrrole substituents was again assigned on the basis of nOe enhancements recorded in DMSO-d_6 (Table 3). In the ^{1}H NMR spectrum of **9**, the H-4 signal at δ 6.96 is shifted upfield relative to the H-4 signals in **3**-**9**, which occur in the range 7.10 to 7.30 ppm. This anomaly was explained by comparing the energy-minimized conformations of compounds in this series, which indicated a unique conformation for **9**, presumably due to steric interactions, in which the H-4 proton is in the ring current of the other pyrrole ring.

Sclerosponges such as *Astrosclera willeyana* have been difficult to classify. However, the discovery of metabolites of the oroidin class, which are typical of the genus *Agelas*, lend support to the assignment of

A. willeyana to the order Agelasida, to which the genus *Agelas* is also assigned. This is only the second report of *Agelas* chemistry in a sclerosponge.[8] Four compounds (**3**, **5**, **7**, and **8**) were evaluated in a battery of high-throughput enzyme and receptor assays but to date no activity worthy of more extensive evaluation has been observed.

EXPERIMENTAL SECTION

General: IR spectra were recorded on a Perkin Elmer 1600 series FT-IR spectrophotometer using AgCl cell and UV spectra were recorded on a Perkin Elmer Lambda 3B spectrophotometer. ^{1}H (500 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on Varian Unity 500 MHz and Bruker WP-200 SY spectrometers, respectively. All spectra are reported in methanol-d_4 or DMSO-d_6 solutions with the chemical shifts in ppm. relative to methanol (δ_H = 3.38, δ_C = 49.0) or DMSO (δ_H = 2.49) as the internal standards. High resolution fast atom bombardment mass spectra (HRFABMS) were run on a ZAB-E4F mass spectrometer, at the Regional Mass Spectrometry Facility, UC Riverside.

Collection, Extraction and Purification: A specimen of the orange sclerosponge *Astrosclera willeyana* (Agelasida, Astroscleridae), collection # POH 93-049 (SIO Benthic Invertebrate Collection # P1159) was collected by hand using SCUBA (- 15 m) from Ant Atoll, Pohnpei, Federated States of Micronesia, and was kept frozen until it was lyophilized and extracted with CH_2Cl_2:MeOH (1:1) at room temperature to afford (5.1 g) of crude extract (SKB). The extract was partitioned between CH_2Cl_2 and MeOH:H_2O (15:85), the aqueous layer was freeze dried and the methanol soluble portion was retained. The CH_2Cl_2 partition was evaporated under reduced pressure, the residue was partitioned between hexane and MeOH:H_2O (90:10), the methanol layer was evaporated and partitioned between CH_2Cl_2 and MeOH:H_2O (3:2). The aqueous layer was concentrated in vacuo and partitioned with ethyl acetate, the aqueous layer was freeze dried and combined with the methanol soluble portion of the original aqueous partition. The methanol soluble material was purified on a C_{18} reverse phase sep-pak using MeOH:H_2O gradient (40:60 - 100:0), desired fractions were subjected to sephadex LH-20 column chromatography with methanol as eluent to afford some pure methylated ageliferins as their hydrogen chloride salts . Final purification of mixed fractions was achieved by reverse phase HPLC on a preparative Dynamax-C_{18} column with a MeOH:H_2O gradient (45:55 - 55:45) containing 0.1% TFA to afford nine compounds: *N*(1′)-methylageliferin (**3**, 12 mg, 0.24% dry wt.), *N*(1),*N*(1′)-dimethylageliferin (**4**, 8 mg, 0.16% dry wt.), *N*(1′)-methylisoageliferin (**5**, 18 mg, 0.36% dry wt.), *N*(1),*N*(1′)-dimethylisoageliferin (**6**, 9 mg, 0.18% dry wt.), *N*(1′)-methyl-2-bromoageliferin (**7**, 15 mg, 0.30% dry wt.), *N*(1′)-methyl-2′-bromoageliferin (**8**, 14 mg, 0.28% dry wt.), *N*(1′)-methyl-2,2′-dibromoageliferin (**9**, 3 mg, 0.06% dry wt.), bromoageliferin (**10**, 4 mg, 0.08% dry wt.), and dibromoageliferin (**11**, 6 mg, 0.12% dry wt.).

***N*(1′)-Methylageliferin (3):** $[\alpha]_D$ = + 40 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{263}$ = 1900, $[\theta]_{247}$ = 2100, $[\theta]_{227}$ = -1000, $[\theta]_{209}$ = 1300; IR (AgCl) 3300 (br), 1685, 1630 cm^{-1}; UV (MeOH) 218 nm (ε 27000), 234 nm (ε 23000, sh), 271 nm (ε 24000); ^{1}H NMR (500 MHz, MeOH-d_4) see Table 1, (500 MHz, DMSO-d_6) δ

1.95 (m, 1H, H-9), 2.07 (m, 1H, H-9′), 2.27 (ddd, 1H, *J* = 16, 9, 2 Hz, H-10′$_{ax}$), 2.61 (dd, 1H, *J* = 16, 5 Hz, H-10′$_{eq}$), 3.13 (m, 1H, H-8′), 3.45 (m, 1H, H-8), 3.53 (m, 1H, H-8′), 3.56 (m, 1H, H-8), 3.72 (br d, 1H, *J* = 8 Hz, H-10), 3.85 (s, 3H, N′-Me), 6.78 (br s, 1H, H-15), 6.89 (d, 1H, *J* = 1.5 Hz, H-4′), 7.01 (dd, 1H, *J* = 3, 1.5 Hz, H-2), 7.08 (m, 1H, H-4), 7.12 (d, 1H, *J* = 1.5 Hz, H-2′), 7.41 (br s, 2H, NH$_2$), 7.43 (br s, 2H, NH$_2$), 8.03 (br t, 1H, *J* = 6 Hz, H-7), 8.36 (br t, 1H, *J* = 6 Hz, H-7′), 11.87 (m, 1H, NH-1), 11.95 (br s, 2H, 2xNH), 12.05 (br s, 1H, NH), 12.22 (br s, 1H, NH); ^{13}C NMR (50 MHz, MeOH-d_4) see Table 2; HMBC (CD$_3$OD) H-9/(C-9′,C-10,C-11), H-9′/(C-8′,C-9,C-10), H-10′$_{ax}$/(C-9,C-8′,C-9′,C-11′,C-15′), H-10′$_{eq}$/(C-9,C-8′,C-9′,C-11′,C-15′), H-8′/(C-6′,C-9′), H-8/(C-6,C-9,C-10,C-9′), H-10/(C-8,C-9,C-11,C-15,C-11′,C-15′), H-8′/(C-9,C-6′,C-9′,C-10′), N-Me/(C-2′,C-5′), H-8/(C-6,C-9,C-10,C-9′), H-4′/(C-2′,C-5′), H-15/(C-11,C-13), H-2′/(C-3′,C-4′,C-5′), H-2/(C-3,C-4,C-5), H-4/(C-2,C-5); FABMS, (int., %) *m/z* = 633, 635, 637 ($[M+H]^+$, 1:2:1, 55%), 431, 433 (1:1, 10%); HRFABMS, found *m/z* = 633.0696, $C_{23}H_{27}N_{10}O_2{}^{79}Br_2$ $[M+H]^+$ requires *m/z* = 633.0685.

***N*(1),*N*(1′)-Dimethylageliferin (4):** $[\alpha]_D$ = + 40 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{260}$ = 1400, $[\theta]_{247}$ = 1800, $[\theta]_{227}$ = -1400, $[\theta]_{210}$ = 1600; IR (AgCl) 3300 (br), 1685, 1630 cm^{-1}; UV (MeOH) 218 nm (ε 28000, sh), 228 nm (ε 26000, sh), 236 nm (ε 23000, sh), 271 nm (ε 23000); ^{1}H NMR (500 MHz, MeOH-d_4) see Table 1; ^{13}C NMR (50 MHz, MeOH-d_4) see Table 2; FABMS (int., %) *m/z* = 647, 649, 651 ($[M+H]^+$, 1:2:1, 30), 431, 433 (1:1, 6); HRFABMS, found *m/z* = 647.0818, $C_{24}H_{29}N_{10}O_2{}^{79}Br_2$ $[M+H]^+$ requires *m/z* = 647.0842.

***N*(1′)-Methylisoageliferin (5):** $[\alpha]_D$ = + 40 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{263}$ = 800, $[\theta]_{247}$ = 1500, $[\theta]_{228}$ = -1000, $[\theta]_{204}$ = 2000; IR (AgCl) 3300 (br), 1685, 1630 cm^{-1}; UV (MeOH) 218 nm (ε 30000), 234 nm (ε 26000, sh), 270 nm (ε 30000); ^{1}H NMR (500 MHz, MeOH-d_4) see Table 1, (500 MHz, DMSO-d_6) δ 1.95 (m, 1H, H-9), 2.07 (m, 1H, H-9′), 2.27 (ddd, 1H, *J* = 16, 9, 2 Hz, H-10′$_{ax}$), 2.61 (dd, 1H, *J* = 16, 4.5 Hz, H-10′$_{eq}$), 3.13 (m, 1H, H-8′), 3.43 (m, 1H, H-8), 3.50 (m, 1H, H-8′), 3.54 (m, 1H, H-8), 3.71 (br d, 1H, *J* = 8 Hz, H-10), 3.85 (s, 3H, N′-Me), 6.25 (d, 1H, *J* = 4.5 Hz, H-3′), 6.76 (br s, 1H, H-15), 6.87 (d, 1H, *J* = 4.5 Hz, H-4′), 7.00 (t, 1H, *J* = 1.5, 1.5 Hz, H-2), 7.07 (m, 1H, H-4), 7.40 (br s, 2H, NH$_2$), 7.42 (br s, 2H, NH$_2$), 8.03 (br t, 1H, *J* = 7 Hz, H-7), 8.39 (br t, 1H, *J* = 6 Hz, H-7′), 11.86 (m, 1H, NH-1), 11.96 (br s, 2H, NH), 12.07 (br s, 1H, NH), 12.24 (br s, 1H, NH); ^{13}C NMR (50 MHz, MeOH-d_4) see Table 2; HMBC (MeOH-d_4) H-10′$_{ax}$/(C-9,C-8′,C-9′,C-11′,C-15′), H-10′$_{eq}$/(C-11′,C-15′), H-8′/(C-6′,C-9,C-9′,C-10′), H-8/(C-6,C-10), H-10/(C-8,C-9,C-11,C-11′,C-15,C-15′), H-8′/(C-10′), H-8/C-6, N-Me/(C-2′,C-5′), H-3′/(C-2′,C-5′), H-15/(C-11,C-13), H-4′/(C-2′,C-3′,C-5′), H-2/(C-3,C-4,C-5), H-4/(C-2,C-5); FABMS, (int., %) *m/z* = 633, 635, 637 ($[M+H]^+$, 1:2:1, 81), 431, 433 (1:1, 14%); HRFABMS, found *m/z* = 633.0653, $C_{23}H_{27}N_{10}O_2{}^{79}Br_2$ $[M+H]^+$ requires *m/z* = 633.0685.

***N*(1),*N*(1′)-Dimethylisoageliferin (6):** $[\alpha]_D$ = + 40 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{263}$ = 800, $[\theta]_{247}$ = 1100, $[\theta]_{228}$ = -1400, $[\theta]_{204}$ = 1600; IR (AgCl) 3300 (br), 1685, 1630 cm^{-1}; UV (MeOH) 218 nm (ε 30000, sh), 228 nm (ε 28000, sh), 234 nm (ε 24000, sh), 272 nm (ε 27000); ^{1}H NMR (500 MHz, MeOH-

d$_4$) see Table 1, (500 MHz, DMSO-*d*$_6$) δ 1.96 (m, 1H, H-9), 2.09 (m, 1H, H-9′), 2.27 (ddd, 1H, *J* = 16, 9, 2.5 Hz, H-10′$_{ax}$), 2.62 (dd, 1H, *J* = 16, 4.5 Hz, H-10′$_{eq}$), 3.11 (m, 1H, H-8′), 3.40 (m, 1H, H-8), 3.51 (m, 1H, H-8′), 3.52 (m, 1H, H-8), 3.72 (br d, 1H, *J* = 8 Hz, H-10), 3.82 (s, 3H, N-Me), 3.86 (s, 3H, N-Me), 6.26 (d, 1H, *J* = 4 Hz, H-3′), 6.76 (br s, 1H, H-15), 6.88 (d, 1H, *J* = 4 Hz, H-4′), 7.02 (d, 1H, *J* = 1.5 Hz, H-4), 7.12 (d, 1H, *J* = 1.5 Hz, H-2), 7.39 (br s, 2H, NH$_2$), 7.41 (br s, 2H, NH$_2$), 8.05 (br t, 1H, *J* = 6 Hz, H-7), 8.40 (br t, 1H, *J* = 6 Hz, H-7′), 11.86 (m, 2H, NH), 12.01 (br s, 1H, NH), 12.15 (br s, 1H, NH); ^{13}C NMR (50 MHz, MeOH-*d*$_4$) see Table 2; HMBC (MeOH-*d*$_4$) H-10′$_{ax}$/(C-8,C-9′,C-11′,C-15′), H-10′$_{eq}$/C-11′, H-8′/(C-6′,C-9′,C-10′), H-8/(C-6,C-10), H-10/(C-9′,C-11,C-11′,C-15,C-15′), H-8′/(C-6′,C-10′), H-8/(C-6,C-10), N-Me/C-2, N′-Me/C-2′, H-3′/(C-2′,C-5′), H-15/(C-11,C-13), H-4′/(C-2′,C-3′,C-5′), H-2/(C-3,C-4,C-5), H-4/(C-2,C-5); FABMS (int., %) *m/z* = 647, 649, 651 ($[M+H]^+$, 1:2:1, 45), 431, 433 (1:1, 8); HRFABMS, found *m/z* = 647.0811, $C_{24}H_{29}N_{10}O_2{}^{79}Br_2$ $[M+H]^+$ requires *m/z* = 647.0842.

***N*(1′)-methyl-2-bromoageliferin (7):** $[\alpha]_D$ = + 50 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{261}$ = 1600, $[\theta]_{247}$ = 2000, $[\theta]_{227}$ = -1000, $[\theta]_{209}$ = 3200; IR (AgCl) 3300 (br), 1685, 1630 cm^{-1}; UV (MeOH) 216 nm (ε 40000), 234 nm (ε 32000, sh), 272 nm (ε 33000); ^{1}H NMR (500 MHz, MeOH-*d*$_4$) see Table 1, (500 MHz, DMSO-*d*$_6$) δ 1.95 (m, 1H, H-9), 2.08 (m, 1H, H-9′), 2.27 (ddd, 1H, *J* = 16, 9, 2 Hz, H-10′$_{ax}$), 2.61 (dd, 1H, *J* = 16, 5 Hz, H-10′$_{eq}$), 3.14 (m, 1H, H-8′), 3.46 (m, 1H, H-8), 3.48 (m, 1H, H-8′), 3.51 (m, 1H, H-8), 3.72 (br d, 1H, *J* = 8 Hz, H-10), 3.84 (s, 3H, N′-Me), 6.77 (br s, 1H, H-15), 6.89 (d, 1H, *J* = 1.5 Hz, H-4′), 7.12 (d, 1H, *J* = 2 Hz, H-2′), 7.14 (br s, 1H, H-4), 7.40 (br s, 2H, NH$_2$), 7.43 (br s, 2H, NH$_2$), 8.04 (br t, 1H, *J* = 6 Hz, H-7), 8.33 (br t, 1H, *J* = 6 Hz, H-7′), 11.94 (br s, 2H, NH), 12.02 (br s, 1H, NH), 12.20 (br s, 1H, NH), 12.74 (m, 1H, NH-1); ^{13}C NMR (50 MHz, MeOH-*d*$_4$) see Table 2; HMBC (CD$_3$OD) H-9/(C-9′,C-10,C-10′), H-10′$_{eq}$/(C-9,C-9′,C-11′,C-15′), H-8/(C-6,C-10), H-10/(C-8,C-9,C-11,C-11′,C-15,C-15′), H-8′/(C-6′,C-10′), N′-Me/(C-2′,C-5′), H-4′/(C-2′,C-5′), H-15/(C-11,C-13), H-2′/(C-3′,C-4′,C-5′), H-4/(C-2,C-5); FABMS, (intensity) *m/z* = 711, 713, 715, 717 ($[M+H]^+$, 1:3:3:1, 49%), 431, 433 (1:1, 9%); HRFABMS, found *m/z* = 710.9759, $C_{23}H_{26}N_{10}O_2{}^{79}Br_3$ $[M+H]^+$ requires *m/z* = 710.9790.

***N*(1′)-methyl-2′-bromoageliferin (8):** $[\alpha]_D$ = + 40 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{263}$ = 1400, $[\theta]_{247}$ = 1500, $[\theta]_{227}$ = -700, $[\theta]_{207}$ = 1800; IR (AgCl) 3300 (br), 1685, 1630 cm^{-1}; UV (MeOH) 220 nm (ε 34000, sh), 234 nm (ε 28000, sh), 272 nm (ε 28000); ^{1}H NMR (500 MHz, MeOH-*d*$_4$) see Table 1, (500 MHz, DMSO-*d*$_6$) δ 1.97 (m, 1H, H-9), 2.09 (m, 1H, H-9′), 2.27 (ddd, 1H, *J* = 16, 9, 2 Hz, H-10′$_{ax}$), 2.61 (dd, 1H, *J* = 16, 5 Hz, H-10′$_{eq}$), 3.19 (m, 1H, H-8′), 3.47 (m, 3H, H-8, H-8, H-8′), 3.74 (br d, 1H, *J* = 8 Hz, H-10), 3.89 (s, 3H, N′-Me), 6.77 (br s, 1H, H-15), 7.02 (m, 1H, H-2), 7.03 (m, 1H, H-4), 7.04 (s, 1H, H-4′), 7.41 (br s, 2H, NH$_2$), 7.42 (br s, 2H, NH$_2$), 7.98 (br t, 1H, *J* = 6 Hz, H-7), 8.40 (br t, 1H, *J* = 6 Hz, H-7′), 11.87 (m, 1H, NH-1), 11.92 (br s, 2H, NH), 12.01 (br s, 1H, NH), 12.19 (br s, 1H, NH); ^{13}C NMR (50 MHz, MeOH-*d*$_4$) see Table 2; HMBC (CD$_3$OD) H-10′$_{ax}$/(C-8′,C-9,C-9′,C-11′,C-15′), H-10′$_{eq}$/(C-8′,C-9,C-9′,C-11′,C-15′), H-8′/(C-6′,C-9′,C-10′), H-8/(C-6,C-10), H-10/(C-8,C-9,C-11,C-11′,C-15,C-15′), H-8′/(C-6′,C-9,C-9′,C-10′), N′-Me/(C-2′,C-5′), H-15/(C-11,C-13), H-4′/(C-2′,C-5′), H-2/(C-3,C-4,C-5), H-

4/(C-2,C-5); FABMS, (intensity) *m/z* = 711, 713, 715, 717 ($[M+H]^+$, 1:3:3:1, 20%), 431, 433 (1:1, 4%); HRFABMS, found *m/z* = 710.9769, $C_{23}H_{26}N_{10}O_2{}^{79}Br_3$ $[M+H]^+$ requires *m/z* = 710.9790.

***N*(1')-methyl-2,2'-dibromoageliferin (9):** $[\alpha]_D$ = +30 ± 20 (c 0.01, MeOH); CD (MeOH) $[\theta]_{263}$ = 300, $[\theta]_{231}$ = -1600, $[\theta]_{209}$ = 1700; IR (AgCl) 3300 (br), 1685, 1620 cm^{-1}; UV (MeOH) 218 nm (ε 35000), 234 nm (ε 28000, sh), 276 nm (ε 31000); ^1H NMR (500 MHz, MeOH-d_4) see Table 1, (500 MHz, DMSO-d_6) δ 2.00 (m, 1H, H-9), 2.11 (m, 1H, H-9'), 2.26 (ddd, 1H, *J* = 16, 9, 2 Hz, H-10'$_{ax}$), 2.54 (dd, 1H, *J* = 16, 4 Hz, H-10'$_{eq}$), 3.33 (m, 1H, H-8), 3.36 (m, 2H, H-8'), 3.50 (m, 1H, H-8), 3.82 (br d, 1H, *J* = 8 Hz, H-10), 3.86 (s, 3H, N'-Me), 6.75 (br s, 1H, H-15), 6.98 (m, 1H, H-4'), 7.02 (m, 1H, H-4), 7.35 (br s, 4H, NH_2), 8.05 (br t, 1H, *J* = 6 Hz, H-7), 8.12 (br t, 1H, *J* = 6 Hz, H-7'), 11.73 (m, 1H, NH), 11.79 (br s, 1H, NH), 11.82 (br s, 1H, NH), 11.99 (br s, 1H, NH), 12.68 (br s, 1H, NH-1); ^{13}C NMR (50 MHz, MeOH-d_4) see Table 2; HMBC (CD_3OD) H-10'$_{ax}$/C-11', H-10'$_{eq}$/(C-9,C-9',C-11',C-15'), H-8'/(C-6',C-9'), H-8/(C-6,C-9,C-9',C-10), H-8'/C-6', H-8/(C-6,C-9,C-9',C-10), H-10/(C-9,C-11,C-15,C-15'), N'-Me/(C-2',C-5'), H-15/(C-11,C-13), H-4'/(C-2',C-5'), H-4/(C-2,C-5); FABMS, (intensity) *m/z* = 789, 791, 793, 795, 797 ($[M+H]^+$, 1:4:6:4:1, 6%); HRFABMS, found *m/z* = 788.8916, $C_{23}H_{25}N_{10}O_2{}^{79}Br_4$ $[M+H]^+$ requires *m/z* = 788.8895.

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References and Notes

1. Braekman, J.-C.; Daloze, D.; Stoller, C.; van Soest, R.W.M. *Biochem. Syst. Ecol.* **1992**, *20*, 417 and references cited therein.
2. Rinehart, K.L. *Pure & Appl. Chem.* **1989**, *61*, 525.
3. Barrow, R.A.; Capon, R.J. *Nat. Prod. Lett.* **1993**, *1*, 243 and references cited therein.
4. Walker, R.P.; Faulkner, D.J.; Van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 6772.
5. Keifer, P.A.; Schwartz, R.E.; Koker, M.E.S.; Hughes, R.G., Jr.; Rittschof, D.; Rinehart, K.L., Jr. *J. Org. Chem.* **1991**, *56*, 2965 and references cited therein.
6. Kobayashi, J.; Tsuda, M.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Nozoe, S. *Tetrahedron* **1990**, *46*, 5579.
7. The unusual negative nOes were also observed by Kobayashi *et al.*[6]
8. Oroidin was reported from *Goreauiella* sp.[2]

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