Tetrahedron Vol. 46, No. 16, pp. 5579-5586, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

AGELIFERINS, POTENT ACTOMYOSIN ATPase ACTIVATORS FROM THE OKINAWAN MARINE SPONGE AGELAS SP.

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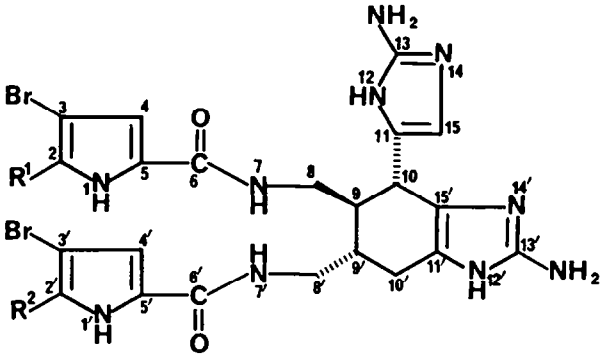
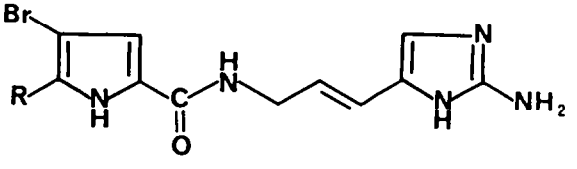
(*Received in Japan* 27 *March* 1990)

Abstract: Three bromopyrrole alkaloids, ageliferin (1), bromoageliferin (2), and dibromoageliferin (3), have been isolated as potent actomyosin ATPase activators from the Okinawan marine sponge Agelas sp. and the structures elucidated on the basis of spectral data, especially two dimensional NMR spectra.

In our studies on bioactive metabolites from marine organisms,[1] we have examined extracts of numerous marine sponges collected in Okinawa and the bioassay-guided purification using isolated vascular smooth muscle resulted in the isolation of several bromopyrroles exhibiting α-adrenoceptor blocking activity[2,3] or antiserotonergic activity.[4,5] Recently we investigated an Okinawan sponge Agelas sp. and isolated three dimeric bromopyrrole compounds as potent actomyosin ATPase activators. The gross structures proved to be identical with ageliferin (1), bromoageliferin (2), and dibromoageliferin (3) isolated previously from the sponges Agelas conifera and A. cf. mauritiana,[6] but the detailed structure elucidation including stereochemistry at C-9' and the position of the bromine atom in bromoageliferin (2) has not been reported. Here we establish the structures of compounds 1 ~ 3 for the first time and describe the full characterization and biological activity of them.



1 $R^1=R^2=H$
2 $R^1=Br, R^2=H$
3 $R^1=R^2=Br$



4 R=Br
5 R=H

The sponge Agelas sp. was collected in Kerama Islands, Okinawa. T methanol extract of the sponge was partitioned between ethyl acetate a water. The ethyl acetate-soluble fraction was applied to a silica gel column with chloroform/1-butanol/acetic acid/water (1.5:6:1:1) to affo an active fraction. This fraction was further separated by HPLC on an column with methanol/water (50:50) containing 0.5% acetic acid to yiel ageliferin (1, 0.02% wet weight), bromoageliferin (2, 0.01%), and dibromoageliferin (3, 0.01%).

The IR spectra of 1 ~ 3 showed similar absorptions at 1660 ~ 1670 cm^{-1} due to the amide carbonyl and the UV spectra of 1 ~ 3 (λ_{max} 270 ~ 278 nm) suggested that all these compounds possess substituted pyrrole chromophores.[2-5] The FABMS spectra of 1 ~ 3 exhibited intense quasi-

molecular ions at m/z 619, 621, and 623 (ca. 1:2:1), m/z 697, 699, 701, and 703 (ca. 1:3:3:1), and m/z 775, 777, 779, 781, and 783 (ca. 1:4:6:4:1), respectively. The intensity ratio of these peaks suggested that compounds 1 ~ 3 contained two, three, and four bromine atoms, respctively. Each molecular formula of 1 ~ 3 was determined to be $C_{22}H_{24}N_{10}O_2Br_2$, $C_{22}H_{23}N_{10}O_2Br_3$, and $C_{22}H_{22}N_{10}O_2Br_4$, respectively, on the basis of high-resolution FABMS analyses in combination with ^1H and ^{13}C NMR spectral data.

The molecular formula of ageliferin (1) is the same as that of sceptrin,[7] a symmetrical dimer of the 2-debromo derivative of oroidin (4)[8] [= hymenidin (5)[4]]. The detailed analysis of the ^1H and ^{13}C NMR data, aided by comparison with those of sceptrin,[7] oroidin (4),[2] and hymenidin (5),[4] revealed that ageliferin (1) possesses an unsymmetrical dimeric structure of hymenidin (5). The ^1H and ^{13}C NMR signals of 1 were firmly assigned on the basis of two-dimensional ^1H-^1H DQF-COSY, HMQC,[9] and HMBC[10] spectra as well as one-dimensional double and triple resonance experiments (Tables 1 and 2). In the ^{13}C NMR of 1 the carbons for two pyrrole moieties including the amide carbonyls (C-2 ~ C-6 and C-2' ~ C-6') were observed as paired signals, viz. each two signals resonated very closely ($\Delta\delta < 0.6$ ppm). The ^{13}C chemical shifts of these signals (C-2 ~ C-6 and C-2' ~ C-6') correlated very well with those of the corresponding signals of hymenidin (5) as shown in Table 2. For the remaining part of the molecule, six sp^2 and six sp^3 carbon signals were observed. By comparison with ^{13}C NMR data of bromopyrroles obtained from marine sponges,[2-5,11] the six sp^2 carbons were assigned to two amino imidazole rings containing the guanidino carbons (150.2 and 149.4 ppm; C-13 and C-13'). The six sp^3 carbons

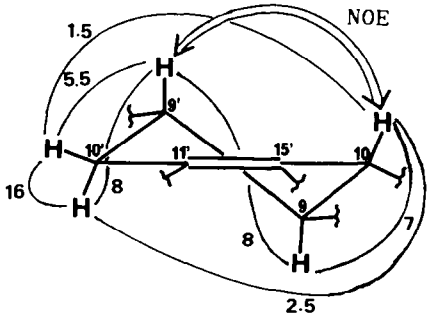


Figure 1. The ^1H-^1H coupling constants (J/Hz) and NOE correlation for the cyclohexene moiety of ageliferin (1).

Table 1. ^{1}H NMR Data of Ageliferins (1 ~ 3)[a]

position	1		2		3	
	δ	J/Hz	δ	J/Hz	δ	J/Hz
2	6.96 d[b]	1.5				
2'	6.97 d[b]	1.5	6.95 brs			
4	6.85 d[c]	1.5	7.02 brs		6.92 brs[d]	
4'	6.94 d[c]	1.5	6.85 brs		7.05 brs[d]	
8a	3.50 dd	14, 5	3.51 dd	14.5, 5	3.55 dd	14.5, 4.5
8b	3.77 dd	14, 4.5	3.74 dd	14.5, 4.5	3.76 dd	14.5, 4
8'a	3.33 dd	14, 4.5	3.30[e]		3.30[e]	
8'b	3.64 dd	14, 3	3.64 dd	14, 3.5	3.66 dd	14, 3
9	2.16 m		2.17 m		2.20 m	
9'	2.27 m		2.27 m		2.30 m	
10	3.83 brd	7	3.83 brd	7.5	3.87 brd	7
10'a[f]	2.48 ddd	16, 8, 2.5	2.48 ddd	16, 8, 2	2.51 dd	16, 8
10'b[g]	2.78 ddd	16, 5.5, 1.5	2.77 ddd	16, 5, 1.5	2.81 dd	16, 5.5
15	6.79 brs		6.81 brs		6.83 brs	

a) in CD_3OD as HCl salts; b-d) Signals may be interchanged; e) Overlapped with the signal of methanol; f) Axial hydrogen; g) Equatorial hydrogen.

Table 2. ^{13}C NMR Data of Ageliferins (1 ~ 3), Oroidin (4), and Hymenidin (5)

position	1[a]	2[b]	3[b]	4[c]	5[d]
2	123.2 d[e]	106.6 s	106.4 s	104.4 s	121.0 d
2'	123.0 d[e]	123.1 d	106.4 s		
3	97.7 s[f]	100.3 s	100.2 s[l]	97.8 s	95.4 s
3'	97.6 s[f]	97.6 s	100.1 s[l]		
4	114.2 d[g]	115.2 d	115.1 d[m]	116.2 d	112.1 d
4'	113.6 d[g]	113.8 d	114.7 d[m]		
5	127.3 s	127.6 s[j]	128.6 s[n]	128.0 s	127.9 s
5'	127.3 s	127.3 s[j]	128.5 s[n]		
6	163.1 s[h]	163.0 s[k]	162.3 s[o]	158.6 s	160.2 s
6'	162.9 s[h]	162.5 s[k]	162.1 s[o]		
8	40.6 t	40.1 t	40.4 t	39.8 d	39.3 t
8'	43.0 t	42.8 t	42.9 t		
9	43.8 d	44.1 d	43.6 d	113.0 d	113.5 d
9'	37.4 d	37.1 d	37.1 d		
10	34.3 d	33.2 d	33.7 d	126.8 d	126.7 d
10'	23.8 t	23.7 t	23.6 t		
11	131.4 s	128.4 s	131.3 s	124.7 s	125.2 s
11'	123.0 s	123.0 s	122.6 s		
13	150.2 s[i]	149.1 s	149.6 s[p]	147.5 s	147.8 s
13'	149.4 s[i]	149.1 s	149.2 s[p]		
15	112.4 d	113.1 d	112.7 d	110.8 d	111.2 d
15'	122.3 s	119.1 s	120.0 s		

a) in CD_3OD as AcOH salts; b) in CD_3OD as HCl salts; c) Reference 2, in DMSO-d_6; d) Reference 4, in DMSO-d_6; e-p) Signals may be interchanged.

(three methylenes and three methines) were corresponding to the C-8 ~ C-10 moiety of hymenidin (5). Interpretation of the DQF-COSY spectrum revealed the cross peaks between H-8a/H-8b, H-8a/H-9, H-9/H-10, H-8'a/H-8'b, H-8'a/H-9', H-9'/H-10'a, and H-10'a/H-10'b and the double and triple resonance experiments clarified the ^{1}H-^{1}H coupling constants shown in Figure 1. These results, in particular, the observation of the homoallylic couplings between H-10/H-10'a and H-10/H-10'b with J-values of 2.5 and 1.5 Hz, respectively, strongly suggested that the presence of a cyclohexene ring attached to one of the two amino imidazole rings. This fact was coincident with the finding that one of the two amino imidazole ring bore an extra substituent (at C-15') which was substantiated by the observations: (i) the DEPT experiment revealed that five of the six sp^2 carbons due to the amino imidazoles were quarternary and (ii) the ^{1}H NMR of 1 showed only one proton signal (6.79 ppm, brs; H-15) ascribable to the proton on the amino imidazole rings. The substituents on the cyclohexene ring at C-9, C-10, and C-9' were deduced to be all equatorial, since the ^{1}H-^{1}H coupling constants for this part were typical for trans-diaxial protons ($J_{9,10}$=7 Hz, $J_{9,9'}$=8 Hz, and $J_{9',10'a}$=8 Hz). In the difference NOE experiment of 1 in CD_3OD solution, an appreciable NOE (3.6%) at H-10 (3.83 ppm) was observed on irradiation of H-9' (2.27 ppm), indicating the 1,3-diaxial relationship between H-10 and H-9'.[12] Additional support for the ring system of 1 was provided by the ^{1}H-^{13}C long-range connectivity data obtained from the HMBC experiment (Experimental Section). The structure of ageliferin was, therefore, concluded to be 1, which was considered to result from [4+2] cycloaddition of two molecules of hymenidin (5) followed by double-bond isomerization.

Molecular formulas of bromoageliferin (2) and dibromoageliferin (3) implied that one hydrogen atom of 1 was substituted by one bromine atom for 2 and two hydrogens by two bromines for 3. The ^{1}H and ^{13}C NMR spectra of compounds 2 and 3 (Table 1 and 2) were essentially identical with those of 1 except for the following points. The ^{1}H NMR of 2 and 3 showed three and two pyrrole ring protons (6.85 ~ 7.05 ppm region), respectively, whereas ageliferin (1) possessed four pyrrole ring protons. These observations are explained well by considering that one and two hydrogens on the pyrrole rings were replaced by one and two bromines for 2 and 3, respectively, being in agreement with their molecular formulas. The substitited positions were deduced to be C-2 positions of the pyrrole rings on the basis of comparison with ^{13}C NMR data of oroidin (4)[2] and hymenidin (5)[4] [C-2: 104.4 ppm (s) for 4 and 121.0 ppm (d) for 5]. Of the two pyrrole rings of compound 2, the hydrogen on C-2 of the pyrrole at-

tached to C-6 was substituted with a bromine atom, while that of the pyrrole attaced to C-6' (H-2') remained unchanged. This fact was reveal by difference NOE experiments in DMSO-d_6 solution of 2. Irradiation of NH-7 (7.94 ppm) produced significant negative NOE (-4%) for the signal o H-4 (7.03 ppm), whereas irradiation of NH-7' (8.09 ppm) yielded NOE (-6% for the H-4' signal (6.88 ppm).[13] The assignment of these ^1H signals we firmly verified by the ^1H-^1H COSY spectrum (Experimental Section). The H-4' signal appeared relatively broad with a long-range coupling with H-2', while the H-4 signal was rather tall and sharp singlet. The signals for NH-7 and NH-7' were clearly discriminated by the COSY cross-peaks in DMSO-d_6 (H/H: NH-7/H-8a, NH-7/H-8b, and NH-7'/H_2-8'). Thus the structur of bromoageliferin and dibromoageliferin were finally assigned to 2 and respectively, the former resulting from [4+2] cycloaddition of oroidin (and hymenidin (5), while the latter constructed from two molecules of oroidin (4).[14]

The actin-myosin system is generally accepted to be involved in muscle contraction and many of other cell-motility activities, and myosi ATPase provides the energy for the mobility events. The ATPase activity of myofibrils from rabbit skeletal muscle[15] was elevated to 150, 190, an 200% of the control value by ageliferin (1, 3 x 10^{-5} M), bromoageliferin (2, 10^{-6} M), and dibromoageliferin (3, 10^{-6} M), respectively. Ageliferi may provide useful chemical tools for the study of the molecular mechanisms of actin-myosin contractile systems, since few substances have bee reported which modulate the ATPase activities of myosin and actomyosin.

Experimental

Optical rotations were determined on a JASCO DIP-360 polarimeter. I.r. spectra were obtained on a Hitachi 260-50 i.r. spectrometer and u.v spectra on JASCO 660 UV/VIS spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on JEOL GX-500, GSX-270, and Bruker AM-500 spectrometers. Mass spectra (FABMS) were obtained on a JEOL HX-100 spectrometer. Wako C-300 silica gel (Wako Pure Chemical) was used for open column chromatog raphy. T.l.c. was carried out on Merck silica gel GF_{254}.

Isolation. The sponge (0.7 kg, wet weight) collected by SCUBA in Kerama Islands, Okinawa, was kept frozen until used. The methanol (1.5 x 2) extract of the sponge was evaporated under reduced pressure to affo the residue (38 g), which was dissolved in a mixed solvent of ethyl acetate (400 mL) and water (400 mL) and then partitioned between ethyl acetate (400 mL x 3) and water (400 mL). The ethyl acetate-soluble materi

(12.4 g) was partially (3.0 g) applied to a silica gel column eluted with chloroform/1-butanol/acetic acid/water (1.5:6:1:1) to give an active fraction (488 mg), part of which (18.6 mg) was further purified by HPLC [YMC-Pack AM-323 ODS, Yamamura Chemical, 10 x 250 mm; flow rate, 2.0 mL/min; UV detection at 254 nm; eluant, methanol/water (50:50) containing 0.5% acetic acid] to yield ageliferins A (1, 4.6 mg, R_t 4.8 min), (2, 3.1 mg, R_t 7.2 min), and C (3, 2.0 mg, R_t 11.4 min).

Ageliferin (1). $[\alpha]_D^{33}$ +15.5° (c 0.11, MeOH); I.r. ν_{max} (KBr) 3380, 1665, and 1620 cm^{-1}; U.v. λ_{max} (MeOH) 204 (ϵ 7300), 222 (7300), 270 (7900), and 393 nm (300); C.d. $[\theta]_{272}$ (2900), $[\theta]_{252}$ (1900), $[\theta]_{232}$ (-5500), and $[\theta]_{212}$ (5700); ^1H NMR (Table 1); ^{13}C NMR (Table 2); ^1H-^{13}C Long-range connectivities obtained by the HMBC spectrum (H/C): H-8a/C-10, H-10'a/C-9', H-10/C-9, H-2,2'/C-3,3'(overlapped cross peaks), H-2'/C-4', H-2/C-4, H-4'/C-2', H-4/C-2, H-4/C-5, H-2'/C-5', H-2/C-5, H-4'/C-5', H-15/C-11, H-15/C-13, H-10/C-15', H-10'a/C-15', H-10/C-11', H-10'b/C-11', H-10'a/C-11', and H-10/C-11; FABMS m/z 623, 621, 619 $(M+H)^+$, 543, and 541; [Found: $(M+H)^+$, 619.0490. Calc. for $C_{22}H_{25}N_{10}O_2{}^{79}Br_2$: M+H, 619.0529].

Bromoageliferin (2). $[\alpha]_D^{33}$ + 8.8° (c 0.08, MeOH); I.r. ν_{max} (KBr) 3300, 1665, and 1620 cm^{-1}; U.v. λ_{max} (MeOH) 204 (ϵ 15000), 222 (12200), 274 (12500), and 396 nm (700); C.d. $[\theta]_{272}$ (2600), $[\theta]_{252}$ (1400), $[\theta]_{232}$ (-6200), and $[\theta]_{212}$ (5000); ^1H NMR (in CD_3OD, Table 1); ^1H NMR (in DMSO-d_6) δ 12.72 (1H, s; NH-1), 12.13 (1H, s; NH-12), 11.92 (2H, br s; NH_2-13), 11.79 (1H, s; NH-1'), 8.09 (1H, t, J=7 Hz; NH-7'), 7.94 (1H, t, J=7 Hz; NH-7), 7.40 (3H, br s; NH-12' and NH_2-13'), 7.03 (1H, s; H-4), 6.98 (1H, br s; H-2'), 6.88 (1H, br s; H-4'), 6.77 (1H, br s; H-15), 3.84 (1H, d, J=8 Hz; H-10), 3.59 (1H, m; H-8b), 3.45 (2H, m; H-8'a and H-8'b), 3.30 (1H, m; H-8a), 2.53 (1H, m; H-10'eq), 2.28 (1H, m; H-10'ax), 2.12 (1H, m; H-9'), and 1.98 (1H, m; H-9); ^{13}C NMR (Table 2); FABMS m/z 703, 701, 699, 697 $(M+H)^+$, 623, 621, 619, 543, 541, 419, and 417; [Found: $(M+H)^+$, 702.9563. Calc. for $C_{22}H_{24}N_{10}O_2{}^{81}Br_3$: M+H, 702.9572].

Dibromogeliferin (3). $[\alpha]_D^{33}$ + 3.0° (c 0.10, MeOH); I.r. ν_{max} (KBr) 3400, 1665, and 1620 cm^{-1}; U.v. λ_{max} (MeOH) 204 (ϵ 12200), 220 (9100), 278 (8200), and 394 nm (300); C.d. $[\theta]_{273}$ (200), $[\theta]_{252}$ (100), $[\theta]_{232}$ (-700), and $[\theta]_{211}$ (700); ^1H NMR (Table 1); ^{13}C NMR (Table 2); FABMS m/z 783, 781, 779, 777, 775 $(M+H)^+$, 703, 701, 699, 697, 623, 621, 619, 528, and 497; [Found: $(M+H)^+$, 776.8925. Calc. for $C_{22}H_{23}N_{10}O_2{}^{79}Br_3{}^{81}Br$: M+H, 776.8719].

Acknowledgements We thank Ms. M. Hamashima, M. Takamatsu, and S. Kosuge for their technical assistance and Mr. Z. Nagahama for his help with sponge collection and Dr. Patricia Bergquist for identification of the sponge.

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