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Anti-Histaminic Activity of Bromopyrrole Alkaloids Isolated from Caribbean *Agelas* Sponges

Francesco Cafieri[a], Rosa Carnuccio[b], Ernesto Fattorusso[a]*, Orazio Taglialatela-Scafati[a]*, Teresa Vallefuoco[a]

[a]Dipartimento di Chimica delle Sostanze Naturali, via D. Montesano, 49, I-80131 Napoli, Italy
[b]Dipartimento di Farmacologia Sperimentale, via D. Montesano, 49, I-80131 Napoli, Italy

Abstract: Four Caribbean *Agelas* sponges (*A. clathrodes, A. conifera, A. dispar, A. longissima*) have been investigated for the alkaloid composition. Along with a series of known bromopyrrole alkaloids, two related novel compounds of this class, dispacamides C (**7**) and D (**8**), were found and their structures determined with spectroscopic methods. All the isolated *Agelas* bromopyrrole alkaloids have been evaluated for the antihistaminic activity on the guinea pig ileum. © 1997 Elsevier Science Ltd.

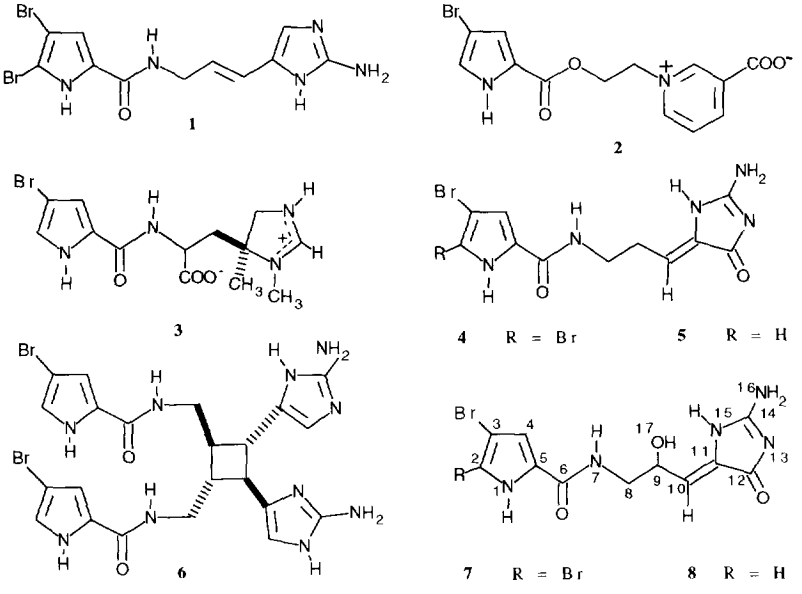
Introduction

In the last 25 years, sponges of the genus *Agelas* (order Agelasida, family Agelasidae) have been extensively investigated yielding a prodigious harvest of new natural compounds, going from α-glycosphingolipids to derivatized terpenoids and bromopyrrole alkaloids, each of these classes including molecules of pharmacological interest. In particular, bromopyrrole alkaloids are very interesting metabolites because of their structural variety and pharmacological activities. These compounds, characterized by a mono- or dibromopyrrole-2-carboxylic acid moiety linked to an imidazolic ring through an aliphatic chain, show bioactivities apparently related to particular structural features. Alkaloids like keramadine,[1] oroidin (**1**),[2] and clathrodine,[3] in which the two heterocyclic nuclei are linked by a linear chain, show serotonergic and/or cholinergic antagonist activities.[4] On the contrary, an α-adrenoceptor blocking activity has been described for those bromopyrrole alkaloids in which the aliphatic chain cycles to form an azepine ring.[5]

During our continuing efforts to identify new biologically active substances from marine sources, we recently focused our interest on *Agelas* sponges, studying the secondary metabolism, and, in particular, the alkaloid composition of four Caribbean species, namely *A. clathrodes*, *A. conifera*, *A. dispar*, and *A. longissima*. From these organisms we isolated a series of structurally related alkaloids, characterized by a short linear chain connecting two heteroaromatic rings: agelongine[6] (**2**, a pyridinium alkaloid that we recently reported as selective antiserotonergic agent), clathramides[7] (**3**), and dispacamides A-B[8] (**4-5**), along with considerable amounts of the known compounds oroidin[2] (**1**), and sceptrin[9] (**6**), previously isolated from other *Agelas* sponges.

Aiming to gain further information on the pharmacological potential of bromopyrrole alkaloids from *Agelas* sponges and considering some structural analogy with known H_1-antagonists, we decided to initiate a study to evaluate the antihistaminic activity of compounds **1-6**. One should remind that some bromopyrrole alkaloids have been previously subjected to antiserotonergic, anticholinergic, antimicrobic, antifouling, and sodium channel blockage activity tests,[10] but none had been evaluated previously for antihistaminic activity.

FAX: 39 81 7486552; E-mail: fattoru@unina.it



In order to obtain sufficient quantities of the bromocompounds to be tested, our *Agelas* specimens have been extracted and, as a result of chromatographic separations, alkaloids **1-6** were isolated as pure products. In addition, two new related metabolites have been found. This paper deals with the structural elucidation of the novel compounds dispacamides C (**7**) and D (**8**), and the results of pharmacological characterization of the antihistaminic properties exhibited by compounds **1-8**.

Chemistry

The four *Agelas* species under investigation, collected by hand along the coasts of Little San Salvador Island, Bahamas, were subjected to identical isolation procedures. Standard sponge preservation and work-up systems were used to obtain methanol extracts. The water soluble portion of the crude extracts was partitioned between H_2O and n-BuOH. The butanolic layer was initially subjected to chromatography over a column packed with reversed phase (RP18) silica gel and eluted with four eluants: H_2O, MeOH/H_2O 1:1, MeOH/$CHCl_3$ 9:1, and $CHCl_3$. Following this procedure, the MeOH/$CHCl_3$ 9:1 fraction is rich in glycosphingolipids, while the fraction eluted with MeOH/H_2O 1:1 was mainly composed of polar alkaloids. This fraction was further purified by a medium pressure liquid chromatography (MPLC) over silica gel, using a system of eluants with a gradient of increasing polarity going from EtOAc to MeOH. Various fractions were thus obtained each containing alkaloids, and a successive purification by HPLC afforded them in a pure form.

Fractions eluted with EtOAc/MeOH 9:1 contained dispacamides A (**4**) and B (**5**); those eluted with EtOAc/MeOH 8:2 afforded oroidin (**1**); the EtOAc/MeOH 7:3 fractions, purified by HPLC (eluant H_2O/MeOH 1:1), gave dispacamides C (**7**) and D (**8**); the fractions eluted with EtOAc/MeOH 6:4 afforded sceptrin (**6**), while more polar fractions, eluted with EtOAc/MeOH 2:8, furnished agelongine (**2**) and clathramides (**3**). The distribution of these alkaloids is not uniform in the examined sponges: for example, clathramides are exclusive of *Agelas clathrodes*, while oroidin and agelongine were found in all the species and in almost the same amounts. Table 1 summarizes the distribution of alkaloids in the examined sponges.

Table 1. Bromopyrrole alkaloid distribution in four Caribbean *Agelas* sponges (expressed as % dry weight)

	A. conifera	*A. dispar*	*A. clathrodes*	*A. longissima*
Sceptrin	8.8	15.5	15.1	2.5
Oroidin	2.1	4.2	2.1	4.1
Agelongine	2.5	2.5	2.3	2.2
Clathramides	0	0	7.2	0
Dispacamides A-C	0.8	3.5	3.2	1.1
Dispacamides B-D	3.5	0.2	0.5	3.3

All the known alkaloids (**1-6**) were identified by comparison of their spectral data with literature values.[6-9]

Characterization of dispacamide C (**7**) commenced with positive FAB mass spectrum, obtained after addition of trifluoromethanesulfonic acid. It showed signals at *m/z* 420, 422 and 424 $[M+H]^+$ (intensity 1:2:1), indicating that **7** contained two bromine atoms, while the diagnostic peaks at M-18 (402, 404, 406), suggested the presence of an OH group. The complete molecular formula $C_{11}H_{11}Br_2N_5O_3$ was established from positive FABHRMS (meas. 419.9338, calc. 419.9307 for $C_{11}H_{11}{}^{79}Br_2N_5O_3$), and was corroborated by NMR data. Further evidence for the existence of an hydroxyl group in the structure of **7** came from the IR (KBr) band at ν_{max} 3580 cm^{-1}, while two amidic carbonyl functionalities were suggested by absorption bands at ν_{max} 1728 (γ-lactam) and 1672 cm^{-1}.

The UV (MeOH) spectrum of **7** strictly resembled that of dispacamide A[8] exhibiting maxima at λ_{max} 227 (ε 11200) and 272 (ε 12500) nm. The close similarity between **7** and dispacamide A (**4**) was also evident from the comparison of their ^1H and ^{13}C NMR spectra, both containing the key resonances of pyrrolic and aminoimidazolonic rings. A detailed analysis of ^1H and ^{13}C NMR spectra of **7**, aided by COSY, HMQC, and HMBC 2D experiments, allowed the assignment of all the proton and carbon signals (see Table 2), and consequently the determination of structure **7** as reported in figure. The COSY spectrum was useful for the elucidation of the spin system of the central chain, while the information arising from HMQC and HMBC spectra confirmed the nature of the identified substructures and allowed the connections among them. In particular, the most diagnostic cross peaks of the HMBC spectrum were observed between H-9 (δ 4.65) and C-11 (δ 135.7), and between C-12 (δ 179.5) and H-10 (δ 5.72), indicating the linkage of the linear chain with the imidazolonic ring, while the cross-peak between C-6 (δ 162.5) and H_2-8 (δ 3.45 and 3.50) confirmed the linkage of C-8 with the pyrrolocarboxamide moiety. Finally, the inspection of the ^1H NMR spectrum recorded in DMSO-d_6 led to the observation of five D_2O-exchangeable signals (reported in Table 2). All these data are in agreement with a structure of a 9-hydroxylated dispacamide A, in which the *Z* stereochemistry of the double bond $\Delta_{10\text{-}11}$ was deduced by the nuclear Overhauser effect between H-9 and NH-15. Specific rotation $[\alpha]_D$ 0.0° (c 0.001 in MeOH) suggested that dispacamide C is a racemate, which was further proved by its CD spectrum, in fact the CD curve was flat between 200 and 400 nm.

Dispacamide D (**8**), obtained in good amounts from *A. conifera* and *A. longissima*, appeared a close structural analogue of **7**. The molecular formula $C_{11}H_{12}BrN_5O_3$, indicated by FABHRMS (meas. 342.0255, calc. 342.0202 for $C_{11}H_{12}{}^{79}BrN_5O_3$), suggested that the sole difference with **7** should be the substitution of a bromine with an hydrogen atom. The position of this substitution (at C-2 on the pyrrole moiety) was confirmed by interpretation of ^1H and ^{13}C NMR (Table 2) spectra and by comparison with data of dispacamide B.

Table 2. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR data of compounds **7** and **8** in CD_3OD.

Position	7		8	
	δC, mult.	δH (mult., int., *J* in Hz)	δC, mult.	δH (mult., int., *J* in Hz)
1-NH		10.35[a] (brs, 1H)		10.50[a] (brs, 1H)
2	106.2,C		122.7,CH	6.94 (d, 1H, 1.7)
3	97.2,C		95.7,C	
4	114.6,CH	6.85 (s, 1H)	112.9,CH	6.82 (d, 1H, 1.7)
5	128.5,C		127.5,C	
6	162.5,C		162.5,C	
7-NH		7.82[a] (brs, 1H)		7.95[a] (brs, 1H)
8a	46.2,CH_2	3.50 (dd, 1H, 13.3, 6.9)	46.1,CH_2	3.48 (dd, 1H, 13.3, 6.9)
b		3.45 (dd, 1H, 13.3, 5.4)		3.43 (dd, 1H, 13.3, 5.4)
9	69.5,CH	4.67 (dt, 1H, 6.9, 5.5)	69.4,CH	4.65 (dt, 1H, 6.9, 5.5)
10	113.0,CH	5.72 (d, 1H, 5.6)	113.0,CH	5.72 (d, 1H, 5.6)
11	135.7,C		135.8,C	
12	179.5,C		179.5,C	
14	168.5,C		168.3,C	
15-NH		7.49[a] (s, 1H)		7.38[a] (s, 1H)
16-NH_2		6.42[a] (s, 2H)		6.45[a] (s, 2H)
17-OH		8.25[a] (s, 1H)		8.22[a] (s, 1H)

a. D_2O exchangeable signals recorded in DMSO-d_6.

Pharmacology

The effect of "linear chain" bromopyrrole alkaloids isolated from Caribbean *Agelas* sponges (**1-8**) has been assessed for antihistaminic activity on histamine-induced contractions of guinea pig isolated ileum.[11]

The histamine agonist (10^{-8}-10^{-4} M) caused a concentration-dependent contraction of the isolated organ. Dispacamide A, at the concentrations 10^{-6} M, $3X10^{-6}$ M, and 10^{-5} M, produced a concentration-dependent depression of the curve for histamine (fig 1a). Likewise, dispacamide B (its monobromo derivative) induced a similar concentration-dependent depression of histamine response curve (data not shown). Furthermore, this inhibition was removed by washing the tissue with fresh medium, indicating that the antagonism produced by dispacamides A and B is reversible. Finally, the concentration-response curves for 5-hydroxytryptamine and acetylcholine were not affected by **4** and **5**, suggesting that their inhibitory effect is specific for the agonist histamine (data not shown).

To obtain a quantitative evaluation of the antagonistic effect of these two compounds, we calculated their apparent affinities expressed as the pD'_2 values (negative logarithm of the molar concentrations of the antagonists which induced a 50% decrease in the maximal response to the agonist) according to Van Rossum.[12] These values were 5.52 ± 0.11 and 5.33 ± 0.08 for dispacamide A and dispacamide B, respectively. The independence of these pD'_2 values from E_A/E_{Amax} confirmed an antagonistic effect of non-competitive type.

Also dispacamides C (**7**) and D (**8**) (tested as racemic mixtures) exhibited a reversible non-competitive antagonism, specific toward histamine receptors; however this activity was mild as compared to dispacamides A and B, with pD'_2 4.48 ± 0.05 and 4.34 ± 0.10, respectively (fig. 1b).

In addition, oroidin (**1**) and sceptrin (**6**) displayed a non-competitive antagonism (fig.1b) with pD'_2 value 4.02 ± 0.11 and 4.24 ± 0.09, respectively, of the same order of that showed by dispacamide C. However, one should bear in mind the fact that oroidin and sceptrin have already been reported as antiserotonergic and anticholinergic agents at the same concentrations, so their antihistaminic effect was not specific. Finally, both agelongine (**2**) and clathramides (**3**) were completely inactive toward histamine receptors, even at millimolar concentrations.

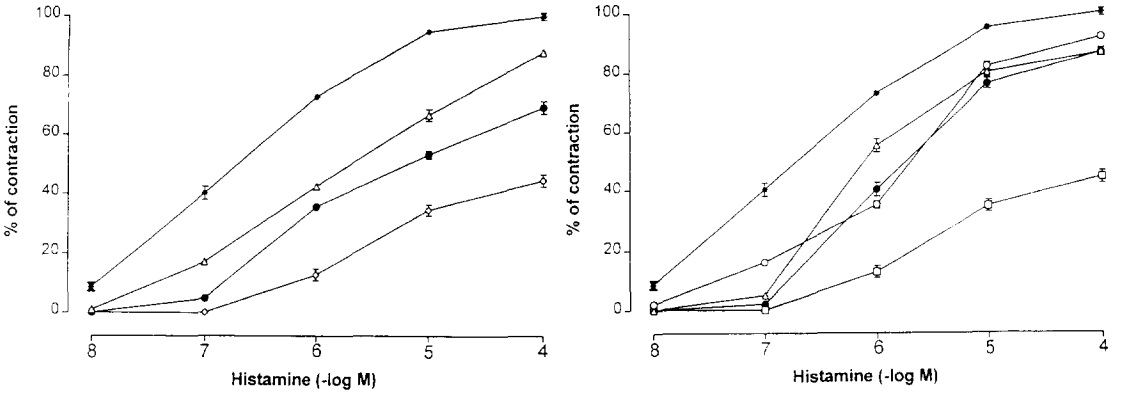


Fig 1. Concentration-response curves depicting histamine-evoked contractions of guinea pig ileum in absence (✳) and in presence of 10^{-6} M (Δ), $3x10^{-6}$ M (●) and 10^{-5} M (✧) dispacamide A (panel a); or 10^{-5} M (O) oroidin, (Δ) sceptrin, (●) dispacamide C, and (□) dispacamide A (panel b). Values are percentage of histamine curve maximum response expressed as means ± s.e.m. (n = 3-4). Differences statistically significant ($P < 0.01$) vs. histamine control.

Conclusions

The results of this preliminary study show that some *Agelas* linear bromopyrrole alkaloids exhibit an antagonistic effect of a non-competitive type toward histamine receptors. This effect is concentration-dependent and occurs in the micromolar range.

In particular, dispacamide A is more potent than all other compounds exhibiting higher affinity for histamine receptors. Oroidin and the [2 + 2] cycloaddition product sceptrin possess a chemical structure rather similar to that of **4** and **5**, however the differences in the alkyl chain and in the imidazole ring cause a clear reduction of pharmacological activity and, above all, a loss of specificity.[13]

On the contrary dispacamides C and D maintain the same nucleus of **4** and **5**, but the insertion of an hydroxylic group in the central chain causes a marked reduction of the antihistaminic activity (fig 1b). This result indicates the importance of the central segment for pharmacological activity, and we hypothesize that this portion of the molecule could interact with hydrophobic groups in the receptor zone, so that the insertion of a polar group achieves the effect of making this interaction worse. In addition, our results indicate that the presence of a bromine atom in the position 2 of the pyrrole ring is not relevant for this pharmacological activity. Finally, agelongine and clathramides, in which the distance between the heteroatom and the heterocyclic ring is reduced to two carbons, show complete loss of antihistaminic activity. These data strengthen the observation that length and functionalization of the central chain are crucial parameters for the antihistaminic action.

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11. Additional data. Experiments were carried out on male Dunkin Hartley guinea pigs weighing 250-300 g. The animals were killed by being stunned and bled via the carotid arteries. The ileum (3-4 cm long) was removed and mounted in 10 ml organ bath containing oxygenated (O_2 95%-CO_2 5%) Tyrode solution (composition in mM: NaCl 136.9; KCl 2.7; $CaCl_2$ 1.8; NaH_2PO4 0.4; $MgCl_2$ 2.1; $NaHCO_3$ 11.9 and glucose 11.1) at 37 °C and was submitted to the constant tension of 1.0 g. Changes of tension produced by agonists were measured using a isotonic transducer (type 7006 Ugo Basile). After a 60-90 min equilibration period, during which the physiological medium was washed out every 15 min, the ileum was contracted with histamine at different concentrations (10^{-8}-10^{-4}), every 5 min and three different concentration-response curves were produced. In these specific experiments some antagonists were also added to the Tyrode solution: propranolol 7.7 mM, atropine 3.8 mM, indomethacin 2.8 mM. The tissue was exposed to a single concentration of compounds **1-8** (dissolved in DMSO/H_2O 1:9) and each experiment was repeated 3 or 4 times. Compounds **1-8** were tested at concentrations 10^{-6}, 3 x 10^{-6} and 10^{-5} M and were added 15 min before the agonist addition. In some experiments other agonists were also used: acetylcholine (10^{-6}–10^{-8} M), 5-hydroxytryptamine (10^{-5}–10^{-7} M).
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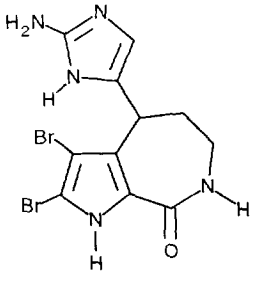
13. Interestingly, the same structural modifications conducting from dispacamide A to oroidin cause a strong increase of the α-blocking activity in the cyclic bromopyrrole alkaloids. In fact hymenin[5] (A, with the nucleus of a cyclized oroidin) is much more active than hymenialdisin[5] (B, possessing the nucleus of a cyclized dispacamide A).



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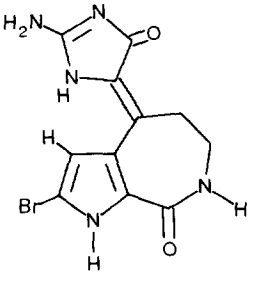

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