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A NOVEL BROMOPYRROLE ALKALOID FROM THE SPONGE *AGELAS LONGISSIMA* WITH ANTISEROTONERGIC ACTIVITY

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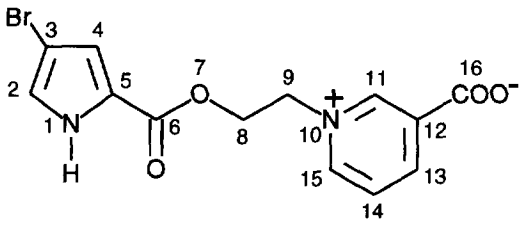
Abstract. Agelongine (**1**), a novel bromopyrrole alkaloid containing a pyridinium ring in place of the imidazole nucleus, usually found in *Agelas* alkaloids, has been isolated from the Caribbean sponge *Agelas longissima*, and its structure established by spectroscopic analysis and chemical degradation. Agelongine exhibited an antiserotonergic activity tested *in vitro* on the rat stomach fundus strip.

The sponges of the genus *Agelas*, commonly found on the Caribbean and Indo-Pacific coral reefs, are a rich source of interesting bromoalkaloids. Structurally, most of them are built up by a 4-bromo- or 4,5-dibromo-pyrrole-2-carboxylic moiety, which is often connected with an aminoimidazole group through an aliphatic segment.

These compounds, as well as some structurally related bromopyrroles isolated from sponges belonging to the genus *Hymeniacidon* (order Halicondrida), show significant pharmacological activities, which seem to be related to some structural features. In particular, an antiserotonergic activity has been found in alkaloids, like keramadine (**2**)[1] and hymenidine,[2] where the two heterocyclic nuclei are joined by a linear chain. On the contrary an α-adrenoceptor blocking activity is peculiar to those compounds, as hymenine[3] (**3**) and debromohymenialdisine,[4] where the aliphatic chain cyclizes to form a seven-membered ring. Finally, an actomyosin ATPase activator function was exhibited by dimeric compounds (ageliferins[5] (**4-6**), oxysceptrin[6]).

As a part of an ongoing search for bioactive metabolites from marine sponges, we found that extracts of the sponge *Agelas longissima* showed an interesting antagonistic activity on serotonergic receptors, as a result of tests performed *in vitro* on the rat stomach fundus strip. Bioassay-guided fractionation of these extracts led to the



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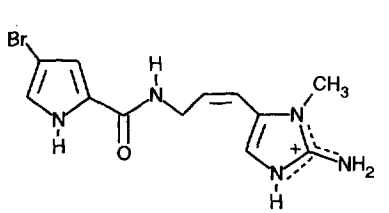
isolation of two active metabolites: the known oroidin[7] (**7**) and the novel bioactive alkaloid, agelongine (**1**), whose structure innovates the standard framework of *Agelas* bromopyrroles in having a pyridinium ring instead of the commonly found imidazole nucleus. In addition **1** contains a quite different central segment where an ester linkage replaces the usual amidic bond. In this paper we report the structural characterization of agelongine and a study of its pharmacological activity.

Specimens of the sponge *Agelas longissima* (order Poecilosclerida, family Agelasidae) were collected in the summer of 1992 along the coasts of San Salvador Island at a depth of 15 m and kept frozen until used. The methanolic extracts were partitioned between *n*-butanol and water. The *n*-butanol soluble fraction was initially separated by MPLC over silica gel (230-400 mesh) by using mixtures of eluants with increasing polarity from EtOAc to MeOH. A further purification of the active fraction (EtOAc/MeOH 1:9) on a C18 reversed phase HPLC column (LiChrocart RP18 5 μ, 250x4 mm) using MeOH/H_2O (1:1) as eluant, yielded pure compound **1** (0.1 % dry weight of the sponge) as an amorphous solid.



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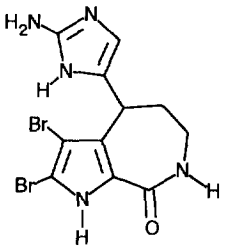

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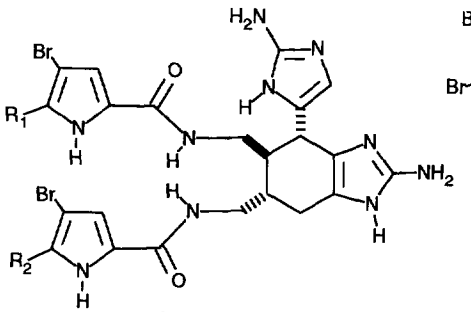
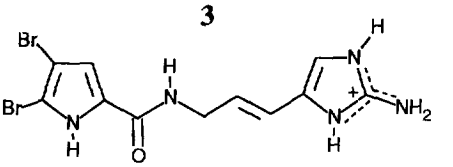


4 $R_1 = R_2 = H$
5 $R_1 = Br$, $R_2 = H$
6 $R_1 = R_2 = Br$



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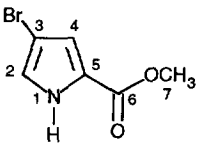

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Table 1. ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectral data for **1**.[a]

Position	δ_C[b]	mult.[c]	δ_H	mult., J [d]	Position	δ_C[b]	mult.[c]	δ_H	mult., J [d]
2	125.6	CH	7.05	d,1.5	11	147.4	CH	9.42	s
3	98.2	C			12	140.2	C		
4	118.5	CH	6.89	d, 1.5	13	147.0	CH	8.99	dt, 7.7, 1.5
5	123.0	C			14	128.8	CH	8.14	dd, 7.7, 6.2
6	161.0	C			15	146.6	CH	9.05	dt, 6.2,1.5
8	63.6	CH_2	4.80	t, 5.5	16	167.0	C		
9	61.7	CH_2	5.07	t, 5.5					

(a) Spectra recorded in CD_3OD. (b) Assignment based on a 2D HMQC experiment. (c) Multiplicity are from a DEPT experiment. (d) Proton coupling constants are given in Hz.

The ionspray[8] mass spectrum of **1** exhibited intense molecular ion peaks at *m/z* 338 and 340 (1:1) (measured mass 337.9910; calculated 337.9903) indicating that **1** was a monobromo compound, compatible with the molecular formula $C_{13}H_{11}N_2O_4Br$, deduced also by ^{1}H and ^{13}C NMR data. The presence of an aromatic ester function and of a carboxylate group was initially suggested by absorption bands in the FT-IR spectrum (KBr) at ν_{max} 1710 and 1646 cm^{-1} respectively, and by ^{13}C NMR singlets at δ 161.0 and 167.0. The ^{13}C NMR spectrum showed also six doublets and three singlets in the sp^2 region (see table 1), indicating the presence of aromatic rings, in agreement with the UV absorption at λ_{max} (MeOH) 262 nm, (log ε = 4.2).

The ^{1}H NMR spectrum exhibited in total eight well separated signals, six of which were in the sp^2 region, while the triplets resulting from two mutually coupled methylenes were visible in the middle region (δ 4.8 and 5.1), thus indicating a deshielding effect probably deriving from two heteroatoms flanked to them.

A detailed analysis of ^{1}H NMR (table 1), achieved by an ^{1}H-^{1}H COSY experiment, illustrated the proton connectivities in **1** and a successive heteronuclear multiple quantum coherence (HMQC) experiment[9] allowed us to assign the resonances of the protonated carbon atoms in the ^{13}C NMR spectrum. The 2D COSY spectrum showed the presence of three distinct spin systems, belonging to two nitrogen-containing heteroaromatic rings, and to the above mentioned -CH_2-CH_2- portion. In particular, the extremely low fields chemical shifts and the multiplicity of signals in the ^{1}H NMR spectrum (δ 8.14, dd; δ 8.99, dt; δ 9.05, dt; δ 9.42, s), well fitting with the data arisen from model compounds[10], allowed us to assign the first nucleus to a β-substituted pyridinium ring. A 2,4-disubstituted pyrrole was suggested by ^{1}H (δ 6.89 and 7.05 long range coupled) and ^{13}C NMR data (δ 125.6, CH; δ 98.2, C; δ 118.5, CH; δ 123.0, C)[11] when compared with other *Agelas* bromopyrrole spectral features.[1-6]

In order to support these hypotheses and to gain informations on the junction among the partial structures, compound **1** was subjected to acid methanolysis using 1N HCl in 80% MeOH. The reaction mixture was evaporeted under nitrogen and partitioned between Et_2O and H_2O. The organic layer was subjected to an HPLC purification using a Si60 LiChrospher (250×4 mm) column with EtOAc/*n*-hexane 8:2 as eluant, yielding pure compound **8**. Its structure was inferred by molecular peaks at *m/z* 203 and 205 (1:1) in a GC-EIMS experiment, and was confirmed by comparing ^{1}H NMR resonances (CD_3OD; δ 7.00, 1H, d; δ 6.85, 1H, d; δ 3.85, 3H, s) with literature data.[12]

Furthermore, useful informations to unambiguously state the complete structure of compound **1** were given by a n.O.e. difference experiment. In particular a strong nuclear Overhauser enhancement of the H-15 and H-11

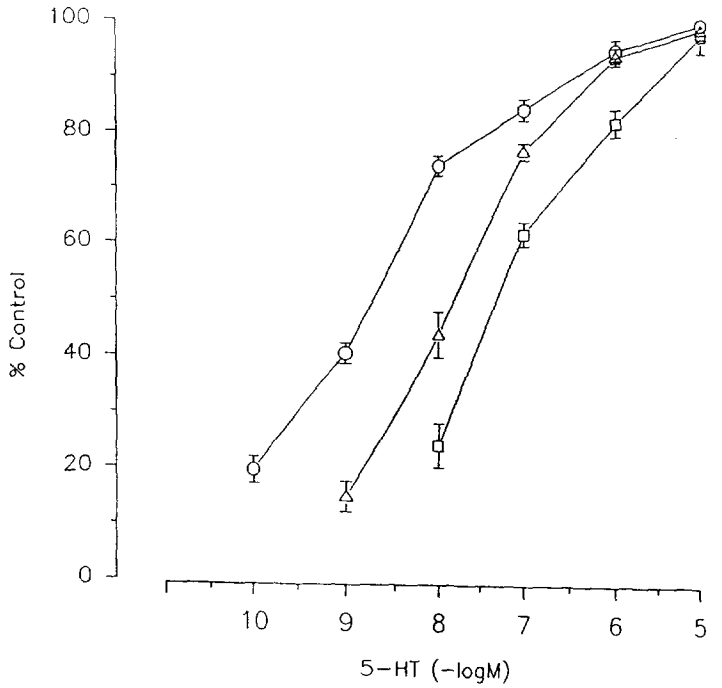

Figure 1. Concentration-response curves depicting the effect of agelongine on 5-HT evoked contractions of rat stomach fundus strip. 5-HT control contractions (O—O); 5-HT + agelongine 10^{-6} M (Δ—Δ); 5-HT + agelongine 10^{-5} M (□—□). Values are percentages of control curve maximum response expressed as means ± s.e.m.(n = 3–6). Differences statistically significant ($P < 0.05$) compared with 5-HT controls.

signals, observed when H_2-9 was irradiated, proved the connection between C-9 and the pyridine nitrogen atom and consequently suggested that the C-8 must be involved in an ester linkage with the pyrrole moiety. Finally the n.O.e. difference results allowed us to assign all the ^1H NMR signals of the pyridinium ring and of the ethylenic bridge.

Compound **1**, subjected to *in vitro* pharmacological test, exhibited an antiserotonergic activity on rat stomach fundus strip.[13] The agonist, 5-hydroxytryptamine (5-HT), caused a concentration-dependent contraction of the isolated organ, while compound **1**, at the concentrations 10^{-6} M and 10^{-5} M shifted the 5-HT concentration-response curve to the right (fig. 1) with a resultant IC_{50} of 8×10^{-5} M. These results indicate a competitive antagonism on the serotonergic receptors of rat stomach fundus. In contrast, the concentration-response curves for histamine, acetylcholine and prostaglandin E_2 were not affected by compound **1**, even at 10^{-5}M (data not shown), suggesting that the inhibitory effect of **1** is specific for the agonist 5-HT. The affinity of compound **1** against serotonergic receptors was estimated by pA_2 value 6.2 ± 0.1 (n = 3), measured with van Rossum method.[14] In addition, the pA_2 value of cyproheptadine on the rat stomach fundus strip was 8.1 ± 0.2 (n = 3) under the same conditions. Furthermore, the inhibitory effect of **1** was completely removed after the tissue had been washed with fresh medium and a second concentration-response curve for the agonist was then constructed, indicating that the antagonism produced by compound **1** is reversible.

This preliminary pharmacological evidence confirms that a specific antagonistic activity on serotonergic receptors is peculiar to those *Agelas* bromopyrroles possessing a linear chain in their structure.[1-2] However our results indicate also that the aminoimidazole nucleus (replaced in **1** by a pyridinium group) is probably not really essential for this activity. In addition some variations in the lenght and geometry of the aliphatic segment linking the two aromatic nuclei appear to be allowed without substantially affecting the bioactivity.

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13. Additional data. Stomach fundus strips from male Wistar rats (200-250 g) were utilized in this experiment to measure the *in vitro* pharmacological activity of compound **1**. The fundus strip 4-6 cm long was mounted vertically in 10 ml organ bath containing a Krebs' solution which was maintained at 37 ^{0}C and continuously gassed with 5% CO_2 in O_2. The composition of the Krebs' solution was (in mM): NaCl 118; KCl 4.8; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 3.3; $NaHCO_3$ 24.9 and glucose 11.1. Isotonic contractions were recorded using a transducer (type 7006 Ugo Basile) with the constant tension of 1.0 g. After a 60 min equilibration period, during which the Krebs' solution was changed several times, the strips were contracted with 5-hydroxy-tryptamine (5-HT) at different concentrations

(10^{-10}–10^{-5} M) at 5 min intervals. In these specific experiments some antagonists were also added to Krebs' solution at following concentrations (mM): mepyramine 0.35; propranolol 7.7; phenoxybenzamine 0.3; atropine 3.8; indomethacin 2.8. The tissue was exposed to the compound **1** (dissolved in DMSO/H_2O 1:9) at the concentrations 10^{-6} and 10^{-5} M for 20 min before the agonist (5-HT) addition. In some experiments other agonists were used: acetylcholine (10^{-6}–10^{-8} M), histamine (10^{-4}–10^{-6} M), prostaglandin E_2 (10^{-6}- 10^{-8} M).

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