Pergamon

Tetrahedron Letters, Vol. 37, No. 20, pp. 3587-3590, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00629-6

Dispacamides, Anti-Histamine Alkaloids from Caribbean *Agelas* Sponges

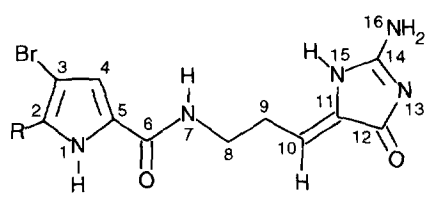
Francesco Cafieri, Ernesto Fattorusso,* Alfonso Mangoni, and Orazio Taglialatela-Scafati

Dipartimento di Chimica delle Sostanze Naturali, via D. Montesano, 49, I-80131 Napoli, Italy

Abstract: Dispacamide **1** and its monobromo derivative **2**, are novel bromopyrrole alkaloids containing an aminoimidazolone moiety. They were isolated from four Caribbean *Agelas* sponges (*A. conifera, A. longissima, A. clathrodes, A. dispar*), and their structures elucidated on the basis of spectroscopic data. Compounds **1** and **2** exhibit remarkably selective antihistamine activity, tested on the guinea pig ileum. Copyright © 1996 Published by Elsevier Science Ltd

A growing collection of intriguing marine natural compounds have been reported in recent years as active leads for new drug development or tools for cell biology.[1,2] As a result of our ongoing studies in the search for new physiologically active metabolites from marine sponges of the genus *Agelas*, we recently obtained agelongine, an inhibitor of serotonergic receptors, from *A. longissima*.[3,4] We wish to report here the isolation and structure determination of two novel alkaloids, dispacamide **1** and its monobromo derivative **2**, obtained by examining four Caribbean *Agelas* species (family *Agelasidae*, order Poecilosclerida), namely *A. dispar*, *A. clathrodes*, *A. longissima*, and *A. conifera*. Compounds **1** and **2** are especially interesting because they show a potent and selective antagonistic activity against histaminergic receptors as a result of tests performed *in vitro* on the guinea pig ileum.

The four *Agelas* sponges under investigation, collected in the Summer 1992 along the coasts of Little San Salvador Island, were subjected to similar isolation procedures. Homogenized fresh organisms were extracted exhaustively with MeOH and the water soluble portion of the crude extract was partitioned between H_2O and n-BuOH.



1 R = Br **2** R = H

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data of Compounds **1** and **2** in CD_3OD.

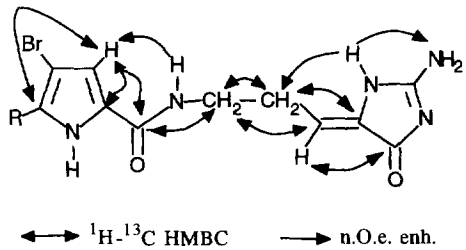
Position	1		2	
	δC, mult.	δH, mult., int., *J* in Hz	δC, mult.	δH, mult., int., *J* in Hz
1-NH		10.43[a] (brs, 1H)		10.52[a] (brs, 1H)
2	106.6,C		122.9,CH	6.94 (d,1H, 1.5)
3	99.7,C		97.4,C	
4	114.4,CH	6.82 (s, 1H)	113.8,CH	6.78 (d, 1H, 1.5)
5	129.0,C		127.1,C	
6	162.1,C		162.8,C	
7-NH		7.75[a] (brs, 1H)		7.88[a] (brs, 1H)
8	39.5,CH_2	3.47 (t, 2H, 6.8)	39.4,CH_2	3.45 (t, 2H, 7.0)
9	28.6,CH_2	2.53 (brq, 2H, 7.5)	28.6,CH_2	2.49 (brq, 2H, 7.3)
10	111.4,CH	5.75 (t, 1H, 8.1)	111.6,CH	5.76 (t, 1H, 8.1)
11	136.9,C		137.0,C	
12	179.1,C		179.1,C	
14	168.3,C		168.3,C	
15-NH		7.41[a] (s, 1H)		7.45[a] (s, 1H)
16-NH_2		5.45[a] (s, 2H)		5.62[a] (s, 2H)

a. D_2O exchangeable signals recorded in DMSO-d_6.

The organic layer was subjected to a medium pressure silica-gel chromatography using an eluant gradient system from EtOAc to MeOH. The EtOAc-MeOH (7:3) fractions were further purified by a C_{18} reversed phase HPLC (LiChrocart RP18 250-4 mm) using H_2O-MeOH 1:1 as eluant. For *A. dispar* and *A. clathrodes* this scheme afforded almost exclusively compound **1** (as an amorphous solid), while *A. conifera* and *A. longissima* exhibited a marked relative predominance of compound **2**.

The FAB mass spectrum (positive ions) of **1**, obtained only after the addition of trifluoromethanesulfonic acid, showed pseudomolecular ion peaks at m/z 404, 406, 408 ($[M+H]^+$) in the ratio 1:2:1, indicating the presence of two bromine atoms. The molecular formula of **1** was determined as $C_{11}H_{11}Br_2N_5O_2$ by HR-FABMS (*m/z* 405.9202 $[M+H]^+$ for $C_{11}H_{11}{}^{79}Br^{81}BrN_5O_2$, calculated 405.9259), in accordance with eight degrees of unsaturation .

The IR spectrum (KBr) of **1** exhibited absorption bands at ν_{max} 1730 and 1675 cm^{-1}, initially suggesting the presence of γ-lactam and amide carbonyl functions, respectively. A detailed analysis of the ^{1}H NMR spectrum of **1** (Table 1), performed with the aid of a 2D ^{1}H-^{1}H COSY experiment, revealed the presence of a -CH_2-CH_2-CH= unit [δ 3.47 (H_2-8, t, J = 6.8 Hz); 2.53 (H_2-9, brq, J = 7.5 Hz); and 5.75 (H-10, t, J = 8.1 Hz)]. Going on, an N-substituted 4,5-dibromopyrrole-2-carboxamide moiety was inferred by ^{13}C NMR pattern of resonances (δ 106.6, C-2; δ 99.7, C-3; δ 114.4, C-4; δ 129.0, C-5; δ 162.1, C-6) strongly reminiscent of those exhibited by other *Agelas* bromopyrrole alkaloids.[5] The presence of such a ring was also supported by UV absorptions at λ_{max} (MeOH) 233 and 274 nm, which are typical for 2-carboxamide substituted pyrrole chromophores.[6] The singlet at δ 6.82 in the ^{1}H NMR spectrum (CD_3OD) was therefore attributed to the sole proton on this heterocyclic nucleus, apart from NH-1 which could be evidenced only in the ^{1}H NMR spectrum recorded in DMSO-d_6 (δ 10.43). In light of the above observations, it remained still unidentified a $C_3H_3N_3O$ subunit, whose carbon resonances were evident in the low-field region of the ^{13}C NMR spectrum at δ 136.9, 168.3, and 179.1. As deduced by a DEPT experiment, the above carbon atoms were unprotonated, thus indicating that the three hydrogen atoms must be attached to heteroatoms. In this regard, the ^{1}H NMR (DMSO-d_6) spectrum was very useful: even if the resonances of H_2-8 and H_2-9 appeared almost completely



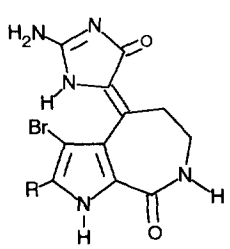
submerged by solvent signals, two D_2O exchangeable signals at δ 7.41 (NH-15) and δ 5.45 (NH_2-16) were visible, in addition to the amidic NH-7 signal at δ 7.75.

Important evidence to define this $C_3H_3N_3O$ subunit came from the heteronuclear multiple-bond ^1H-^{13}C HMBC NMR spectrum. The most diagnostic ^3J cross-peaks were observed between δ 2.53 (H_2-9) and δ 136.9 (C-11), and between δ 5.75 (H-10) and δ 179.1 (C-12), indicating the linkage of C-10 with the sp^2 carbon atom C-11. This HMBC evidence and the n.O.e. enhancement of H_2-9 observed after irradiation on NH-15 (δ 7.41), allowed also to locate a carbonyl (C-12) and an NH group (NH-15), respectively, in the two positions adjacent to the C-11. In addition, the above reported n.O.e. difference measurement also revealed the *Z* stereochemistry of the double bond $\Delta^{10\text{-}11}$. Furthermore, the observed dipolar coupling between NH_2-16 (δ 5.45) and NH-15 furnished the decisive indication to identify the still unassigned substructure as an aminoimidazolone ring linked to the remaining part of the molecule through an exocyclic double bond. With the assembly of the above described partial units, achieved by the whole network of correlations in the HMBC experiment, it was completely defined the structure of compound **1**. However, it should be pointed out that the reported tautomeric form was inferred by performing ^1H NMR experiments in DMSO-d_6, and that the predominance of another tautomer in a different solvent cannot be excluded.

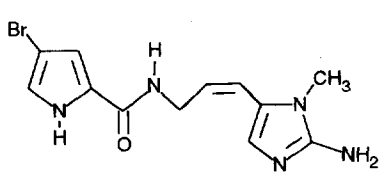
Compound **2** is closely related to **1**, so their structural differences were easily deduced on the basis of a comparison between IR, UV, and, above all, ^1H and ^{13}C NMR data (Table 1). The molecular formula $C_{11}H_{12}BrN_5O_2$ was also indicated by HR-FABMS (*m/z* 326.0201 $[M+H]^+$ for $C_{11}H_{12}{}^{79}BrN_5O_2$, calculated 326.0174).

Dispacamide **1** and monobromodispacamide **2** are novel bromopyrrole alkaloids which differ from oroidin[7] (the first alkaloid of the series) both in an isomerization of the double bond position and in an oxidation of the imidazole ring, which becomes an unusual imidazolone. Moreover, their widespread occurrence in relatively high amounts (1-5 % of the butanolic extracts) suggests for **1** and **2** a pivotal role in the living organism, possibly also as progenitors for the biosynthesis of other bromopyrrole alkaloids. For instance, the α-adrenoceptor blocking hymenialdisins[8] (also reported as "yellow compounds") **3** could be considered cyclic derivatives of dispacamides.

The plain structural resemblances of **1** and **2** with alkaloids like keramadine[9] **4** (that we also found in small amounts in *Agelas dispar* and *A. clathrodes*), or clathrodine[10] (absent in our specimen of *A. clathrodes*), which display an antagonistic activity against serotonergic and cholinergic[11] receptors, induced us to test **1** for



3



4

this aim. Surprisingly, compounds **1** and **2** were found fully inactive both as anticholinergic and antiserotonergic agents, even at millimolar concentrations. On the other hand, dispacamide **1**, exhibited a remarkable antihistamine activity, tested on the isolated guinea pig ileum.[12] In particular, the 1 μM response of histamine was almost completely abolished, and in a reversible manner, by a 3 μM solution of **1**. This result is particularly interesting if we consider that compounds **1** and **2** appear not to possess all the structural features which are currently used as guidelines for the synthesis of antihistamine drugs.[13]

ACKNOWLEDGEMENTS

This work was sponsored by CNR (contr.1994 n° 1598 CT03) and by M.U.R.S.T. (40% and 60%). We thank Prof. W. Fenical for giving us the opportunity to participate in an expedition to the Caribbean Sea, during which the sponges *A. longissima, A. clathrodes, A. dispar, A.conifera* were collected, and Prof. M. Pansini (Istituto di Zoologia, Università di Genova, Italy) for identifying these organisms. Mass, UV, IR, and NMR spectra were performed at the "Centro di Ricerca Interdipartimentale di Analisi Strumentale", Università di Napoli. Pharmacological experiments were performed at "Dipartimento di Farmacologia Sperimentale", Università di Napoli, with the helpful collaboration of Prof. Rosa Carnuccio.

REFERENCES AND NOTES

1. Rinehart, K.; Holt, T.; Fregeau, N.; Keifer, P.; Wilson, C.; Perun, T.; Sakai, R.; Thompson, A.; Stroh, J.; Shield, L.; Seigler, D.; Li, H.; Martin, D.; Grimmelikhuijzen, C.; Gade, G. *J. Nat. Prod.* **1990**, *53*, 771.
2. Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, *93*, 1897.
3. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O.; Carnuccio, R. *BioMed. Chem. Lett.* **1995**, *8*, 799.
4. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1995**, *43*, 7893.
5. Foley, L. H.; Habgood, G. J.; Gallagher, K. S. *Mag. Res. Chem.* **1988**, *26*, 1037.
6. Jaffe, H. H.; Orchin, M. *Theory and Application of UV spectroscopy*; Wiley: New York, 1962, p. 350-351.
7. Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *Chem. Comm.* **1971**, 1129.
   Garcia, E.; Benjamin, L.; Fryer, I. *J. Chem. Soc. Chem. Comm.* **1973**, 78.
8. Kitagawa, I.; Kobayashi, M.; Kitanaka, K.; Kido, M.; Kyogoku, Y. *Chem. Pharm. Bull* **1983**, *31*, 2321.
   Cimino, G.; De Rosa, S.; De Stefano, S.; Mazzarella, L.; Puliti, R.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 767.
9. Nakamura, H.; Ohizumi, Y.; Kobayashi, J.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 2475.
10. Morales, J. J.; Rodriguez, A. D. *J. Nat. Prod.* **1991**, *54*, 629.
11. Rosa, R.; Silva, W.; Escalona de Motta, G.; Rodriguez, A. D.; Morales, J. J.; Ortiz, M. *Experientia* **1992**, *48*, and references cited therein.
12. Ileum from guinea pigs (weighing 250 g) was mounted vertically in 10 ml organ bath containing a Tyrode solution, which was maintained at 37 °C and continuously gassed with 5% CO_2 in O_2. Isotonic contractions were recorded using a transducer (type 7006 Ugo Basile) with the constant tension of 1.0 g. In the reported experiment, repeated four times, some antagonists were also added to the Tyrode solution: propranolol 7.7 mM, atropine 3.8 mM, indomethacin 2.8 mM.
13. Naruto, S.; Motoc, I.; Marshall, G.R. *Eur. J. Med. Chem.* **1985**, *20*, 529.

(*Received in UK* 29 *February* 1996; *revised* 28 *March* 1996; *accepted* 3 *April* 1996)