Pergamon

Tetrahedron Letters 41 (2000) 9917–9922

TETRAHEDRON LETTERS

Two novel pyrrole-imidazole alkaloids from the Mediterranean sponge *Agelas oroides*

Ernesto Fattorusso and Orazio Taglialatela-Scafati*

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

Received 21 July 2000; accepted 4 October 2000

Abstract

The novel pyrrole-imidazole alkaloids cyclooroidin (**7**) and taurodispacamide A (**8**) have been isolated from the Mediterranean sponge *Agelas oroides*, and their structures established from spectroscopic data. Although both these alkaloids may be conceived as derivatives of the $C_{11}N_5$ skeleton of the known oroidin (**1**), remarkably cyclooroidin (**7**) possesses the unprecedented N1/C9 connection. Taurodispacamide A (**8**) exhibited a good antihistaminic activity, tested on the isolated guinea pig ileum.

Keywords: marine metabolites; biologically active compounds; alkaloids; stereochemistry.

Pyrrole-imidazole alkaloids continue to excite the interest of marine natural product chemists because of their structural diversity and interesting biological activities. Although these molecules are exclusively elaborated by the secondary metabolism of marine sponges belonging to the orders Agelasida, Axinellida, and Halichondrida, more than 50 pyrrole-imidazole alkaloids have now been isolated.[1] Structurally they can be conceived as derivatives of the $C_{11}N_5$ skeleton of oroidin[2] (**1**) through: (i) isomerization of the double bond and/or oxidation/reduction; (ii) dimerization; and (iii) cyclization. In particular, five modes of oroidin cyclization (Fig. 1) have been discovered in nature,[1] and we propose that they may be classified based on the oroidin atoms involved in the linkage formation. Accordingly, alkaloids presently characterized can be classified in the following five groups: C4/C10 (e.g. hymenialdisine,[3] **2**); N1/C12+N7/C12 (e.g. dibromoagelaspongine,[4] **3**); N1/C12+N7/C11 (e.g. dibromophakellin,[5] **4**); C4/C12+N7/C11 (e.g. dibromoisophakellin,[6] **5**); and N1/C9+C8/C12 (e.g. agelastatin,[7] **6**).

* Corresponding author. Tel: +39-081-678509; fax: +39-081-678552; e-mail: scatagli@unina.it

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01764-0

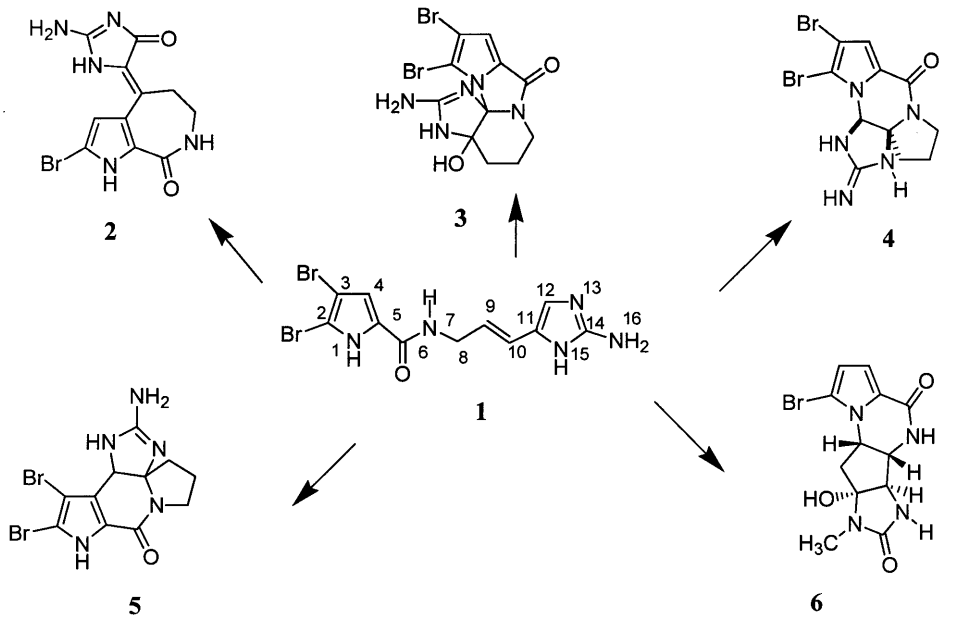
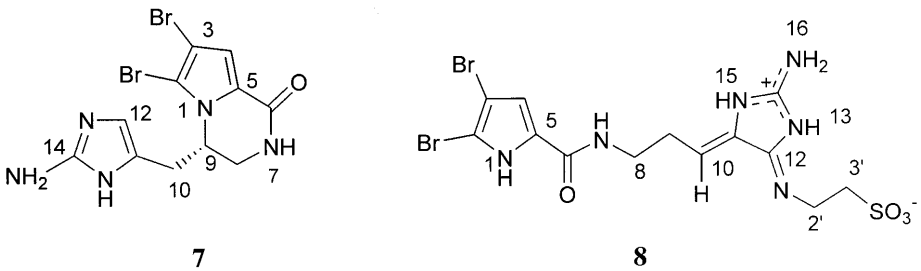


Figure 1. A schematic view of the five cyclization modes of the oroidin skeleton



Our research group has been engaged in the study of secondary metabolites of *Agelas* sponges collected in the Caribbean Sea for a long time, leading to the isolation of several novel bromopyrrole alkaloids, such as the antihistaminic dispacamides[8,9] (whose complete synthesis has been recently reported by two different groups).[10,11] As a part of a project aimed at the identification of lead compounds for the development of drugs active on the peripheral nervous system, we have now selected Agelasida sponges of the Mediterranean Sea as possible sources of candidates for the pharmacological evaluation. In this paper we report the isolation of two novel pyrrole-imidazole alkaloids, cyclooroidin (**7**) and taurodispacamide A (**8**), from the Mediterranean sponge *Agelas oroides*. Remarkably, if we consider data reported in Fig. 1, the simple N1/C9 connection of compound **7** must be considered a novel mode of cyclization of the oroidin skeleton.

Exhaustive methanol extraction of the sponge *A. oroides* (order Agelasida, family Agelasidae) collected in the bay of Naples, afforded a brown-colored crude extract which was partitioned between H_2O and *n*-BuOH. The organic material was initially subjected to MPLC over a C_{18} stationary phase, and then alkaloid-containing fractions were re-chromatographed by silica gel MPLC using an eluant gradient system from EtOAc to MeOH. Preliminary pharmacological

tests were carried out on selected fractions eluted with MeOH–EtOAc 6:4 to 8:2. These were further purified by C_{18} reversed-phase HPLC (LUNA C18 250×4 mm, gradient system from H_2O–MeOH 6:4 to 1:1), yielding the known dispacamides A and B and the novel alkaloids cyclooroidin (**7**, 9×10^{-4}, dry weight) and taurodispacamide A (**8**, 3×10^{-4}), in the pure form as pale yellow amorphous solids.

The FAB mass spectrum (positive ions) of **7** showed pseudomolecular ion peaks at m/z 388, 390, 392 ($[M+H]^+$) in the ratio 1:2:1, clearly indicating the presence of two bromine atoms. The molecular formula of **7** was determined as $C_{11}H_{11}Br_2N_5O$ by HR-FABMS (m/z 389.9559 $[M+H]^+$ for $C_{11}H_{11}{}^{79}Br^{81}BrN_5O$, calculated m/z 389.9518), implying 8 degrees of unsaturation. The IR (KBr) spectrum of **7** exhibited absorption bands at ν_{max} 3420 and 1700 cm^{-1}, suggesting the presence of a NH_2 group and a conjugated δ-lactam carbonyl function, respectively. The ^1H NMR spectrum (Table 1, CD_3OD) of **7** showed two 1H singlets at δ 6.97 and 6.32, and a series of resonances that a COSY experiment allowed us to attribute to a CH_2–CH–CH_2 spin system [δ 2.81 (H_2-10, bd, $J=6.3$ Hz), 4.61 (H-9, m); 3.78 (H-8a, dd, $J=13.6$, 3.5 Hz), and 3.55 (H-8b, dd, $J=13.6$, 1.2 Hz)]. The presence of an *N*-substituted 4,5-dibromopyrole-2-carboxamide moiety was indicated by both the ^{13}C NMR signals at δ 111.4 (C-2), 99.3 (C-3), 115.1 (C-4), and 128.5 (C-5), and the UV absorptions at λ_{max} (CH_3CN) 235 and 276 nm.[12] The singlet at δ 6.97 was attributed as the sole proton on this skeleton due to the HMQC spectrum, which associated it with the ^{13}C NMR signal at δ 115.1. An amino-imidazole moiety was identified as the remaining $C_3H_4N_3$ fragment, mainly based on the comparison between the ^1H and ^{13}C NMR data obtained and those reported in the literature for other *Agelas* pyrrole-imidazole alkaloids.[2,13]

Table 1
^{13}C and ^1H NMR data of cyclooroidin (**7**) and taurodispacamide A (**8**) in CD_3OD

Pos.	**7**			**8**		
	δC, mult.	δH, mult., J in Hz	HMBC	δC, mult.	δH, mult., J in Hz	HMBC
1					11.85, br.s[a]	
2	111.4, C		4, 9	106.2, C		4
3	99.3, C		4	99.9, C		4
4	115.1, CH	6.97, s		114.5, CH	6.82, s	
5	128.5, C		9	128.8, C		
6	163.5, C		8	161.9, C		8
7					8.00, br.s[a]	
8a	45.6, CH_2	3.78, dd, 13.6, 3.5	10	39.5, CH_2	3.48, t, 7.5	9, 10
8b		3.55, dd, 13.6, 1.2				
9	57.9, CH	4.61, m	8, 10	29.1, CH_2	2.64, q, 7.8	8, 10
10	33.2, CH_2	2.81, bd, 6.3		116.6, CH	5.89, t, 7.9	
11	132.9, C		9, 12	141.2, C		9, 10
12	114.8, CH	6.32, s	10	170.4, C		2′, 10
13					9.55, s[a]	
14	153.9, C			168.8, C		
15					9.05, s[a]	
16					5.65, s[a]	
2′				40.4, CH_2	3.86, t, 7.5	3′
3′				50.4, CH_2	3.15, t, 7.5	2′

[a] D_2O exchangeable signals recorded in DMSO-d_6.

The 2D HMBC spectrum was a very useful tool in order to interconnect the above three partial structures, and also to infer an unambiguous assignment of all the carbon resonances. Among the complete series of correlations (reported in Table 1), the key cross-peaks H-9/C-2, H-9/C5, and H_2-8/C-6 allowed us to build up the pyrroloketopiperazine nucleus, while cross-peaks H-9/C-11 and H_2-10/C-12 indicated that this nucleus must be connected to the imidazole ring through the methylene at position 10. Supporting evidence for the above structural framework was provided by the diagnostic peaks at m/z 292, 294, and 296 (ratio 1:2:1) in the FABMS, corresponding to the loss of the methylenaminoimidazole ($C_4H_6N_3$) moiety from the pseudomolecular ion. The basic structure of cyclooroidin (**7**) was therefore completely defined. It corresponds to the product of formation of a bond between N1 and C9 of oroidin, a cyclization mode found only in the agelastatin (**6**)[7] skeleton, where, however, the additional linkage of C-8 with C-12 is present.

The negative optical rotation $[\alpha]_D$ −12 (c 0.02 in MeOH) exhibited by compound **7** suggests that its biosynthesis must occur in a stereoselective way. The absolute stereochemistry at the sole asymmetric carbon C-9 of **7** was determined by following the approach recently applied to longamide,[14] a simpler alkaloid from *A. longissima* containing an OH group instead of the methylenaminoimidazole moiety. This approach is based on the comparison of chiroptic properties of the molecule with those of a suitable model compound. As for longamide, we have chosen dibromophakellin (**4**)[5] for this aim, whose structure, confirmed by X-ray, possesses the same pyrrolelactam chromophore as compound **7**.

The UV spectrum (CH_3CN) of **7** exhibited three strong absorptions at λ_{max} 276 (ε 15 100), 235 (ε 16 100), and 209 (ε 16 200), while its CD spectrum revealed negative Cotton effects at 238 nm ($\Delta\varepsilon$ −6.2) and 208 nm ($\Delta\varepsilon$ −15.5). Interestingly, these spectroscopic data appeared to be almost identical to those reported for dibromophakellin (**4**).[5]

The conformational behavior of compound **7** has been explored by molecular dynamic analysis in the CHARMm force field (1000 K simulations, heating period 1.2 ps, dynamic simulation 30 ps) generating 100 conformations. After minimization in the same force field, the CHARMm energy of the half-chair conformer of **7** possessing H-9 in the quasi-equatorial position was found to be significantly lower than that of all the other conformers. This result is in perfect agreement with the ^{1}H NMR spectrum of **7**, which showed small coupling constants of H-9 with both H-8a and H-8b.

As previously reported,[14] a similar distorted half-chair conformation with the nitrogen atom at C-9 in the quasi-equatorial position is predominant for the rigid polycyclic structure of **4**. As we have already verified for longamide, in the lowest-energy conformations of both **7** and **4**, the pyrrolelactam chromophore is not planar, but slightly skewed around the C-5/C-6 bond, giving rise to an inherently chiral chromophore. In particular, in compound **7** with the *S* configuration at C-9, the O–C6–C5–C4 dihedral angle is about −5° (+5° for the 9*R* enantiomer), while in compound **4** the same angle is −7°. In conclusion, the consistency of CD data for **7** and **4** indicates the same chromophore helicity for both compounds, and therefore the *S* configuration has been assigned to the asymmetric carbon C-9 in compound **7**.

The molecular formula of taurodispacamide A (**8**) was determined as $C_{13}H_{16}Br_2N_6O_4S$ by HRESIMS [(negative ions), m/z 510.9477 $(M-H)^-$, calculated m/z 510.9492], measured on the central peak of the 1:2:1 triplet at m/z 509, 511, and 513. The IR spectrum of **8** suggested the presence of NH (ν_{max} 3420 cm^{-1}), amide carbonyl (ν_{max} 1725 and 1690 cm^{-1}), and sulfonate groups (ν_{max} 1210 and 1040 cm^{-1}), while the UV absorptions at 230 and 270 nm were easily attributed to a substituted pyrrole chromophore.[12] The ^{1}H and ^{13}C NMR data of **8** (Table 1)

appeared practically identical to those of dispacamide A,[8] with only two exceptions: (i) an upfield shift of C-12 in the ^{13}C NMR spectrum (δ 170.4 instead of δ 179.1); (ii) the presence of two additional signals in both ^{1}H (two mutually coupled triplets at δ 3.15 and 3.86) and ^{13}C NMR spectra (two CH_2 at δ 50.4 and 40.4), consistent with those reported in the literature for taurine residues.[15,16]

The whole skeleton of taurodispacamide A (**8**) and the location of the taurine moiety were secured by analyzing the 2D NMR data. In particular, having associated all the proton signals to those of the relevant carbon atoms through the HMQC spectrum, inspection of the HMBC data allowed us to confirm the presence of a dispacamide skeleton and to link unambiguously the taurine residue to the C-12 (cross peak H_2-2′/C-12). The whole series of HMBC cross peaks is reported in Table 1.

The ^{1}H NMR spectrum of **8**, measured in DMSO-d_6 (Table 1), showed the presence of five exchangeable signals and revealed very useful information relevant to the structure of taurodispacamide A. In particular, the NOE contact between NH-15 (δ 9.05) and CH_2-9 indicated the *Z* stereochemistry of the double bond, while the dipolar couplings of the 2H-integrating signal at δ 5.65 (NH_2-16) with those of NH-15 and NH-13 allowed the complete assignment of the proton NMR signals of the aminoimidazolone cation.

Taurodispacamide A (**8**) is a novel alkaloid possessing a taurine residue attached to the imidazole ring. This feature is quite uncommon, other examples of taurine-containing pyrrole-imidazole alkaloids being only mauritiamide A[15] (from *Agelas mauritiana*) and tauroacidins[16] (from *Hymeniacydon* sp.), endowed with tyrosine kinase inhibitory activity.

Compounds **7** and **8** have been tested for anticholinergic, antiserotonergic, and antihistaminic activity on the isolated guinea pig ileum. Taurodispacamide A (**8**) exhibited a good antihistaminic activity, and in particular, the 0.1 μM response of histamine was almost completely abolished, in a reversible manner, by a 10 μM solution of **8**. On the contrary, cyclooroidin (**7**) showed no activity on the isolated organ. More detailed description of the pharmacological results will be reported elsewhere.

Acknowledgements

This work is the result of a research co-sponsored by Regione Campania, legge 41/94, annualità '97 'Impiego di metaboliti azotati da invertebrati marini come composti lead per la progettazione di farmaci attivi sul sistema nervoso' and by M.U.R.S.T., PRIN 'Chimica dei Composti Organici di Interesse Biologico', Rome, Italy. Mass, IR, and NMR experiments were performed at 'Centro di Ricerca Interdipartimentale di Analisi Strumentale', Università di Napoli 'Federico II'.

References

1. Lindel, T.; Hoffmann, H.; Hochgürtel, M. In *Chemistry of Marine Pyrrole-Imidazole Alkaloids*; Diederichsen, U.; Lindhorst, T. K.; Westermann, B.; Wessjohann, L. A., Eds. Bioorganic chemistry. John Wiley: New York, 1999; pp. 8–17.
2. Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129–1130.
3. Kitagawa, I.; Kobayashi, M.; Kitanaka, K.; Kido, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1983**, *31*, 2321–2328.

4. Fedoreyev, S. A.; Ilyin, S. G.; Utkina, N. K.; Maximov, O. B.; Reshetnyak, M. V.; Antipin, M. Y.; Struchkov, Y. T. *Tetrahedron* **1989**, *45*, 3487–3492.
5. Sharma, G.; Magdoff-Fairchild, B. *J. Org. Chem.* **1977**, *42*, 4118–4124.
6. Fedoreyev, S. A.; Utkina, N. K.; Ilyin, S. G.; Reshetnyak, M. V.; Maximov, O. B. *Tetrahedron Lett.* **1986**, *27*, 3177–3180.
7. D'Ambrosio, M.; Guerriero, A.; Debitus, C.; Ribes, O.; Pusset, J.; Leroy, S.; Pietra, F. *J. Chem. Soc., Chem. Commun.* **1993**, 1305–1306.
8. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1996**, *37*, 3587–3590.
9. Cafieri, F.; Carnuccio, R.; Fattorusso, E.; Taglialatela-Scafati, O.; Vallefuoco, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2283–2288.
10. Lindel, T.; Hoffmann, H. *Tetrahedron Lett.* **1997**, *38*, 8935–8938.
11. Olofson, A.; Yakushijin, K.; Horne, D. A. *J. Org. Chem.* **1998**, *63*, 1248–1253.
12. Jaffe, H. H.; Orchin, M. *Theory and Application of UV Spectroscopy*; Wiley: New York, 1962; pp. 350–351.
13. Williams, D. H.; Faulkner, D. J. *Tetrahedron* **1996**, *52*, 5381–5390.
14. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1995**, *36*, 7893–7896.
15. Jimenez, C.; Crews, P. *Tetrahedron Lett.* **1994**, *35*, 1375–1378.
16. Kobayashi, J.; Inaba, K.; Tsuda, M. *Tetrahedron* **1997**, *53*, 16679–16682.