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A new cytotoxic tambjamine alkaloid from the Azorean nudibranch *Tambja ceutae*

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ABSTRACT

The chemical investigation of Azorean nudibranch mollusk *Tambja ceutae* led us to isolate a new member of the tambjamine family, tambjamine K (**1**). The bryozoan *Bugula dentata*, prey of the nudibranch, was also analyzed and found to contain compound **1** in very small amounts together with known blue pigment **2** and tambjamines A (**3**) and B (**4**). The structure of tambjamine **1** was elucidated by the interpretation of the spectroscopic data as well as by the comparison with related compounds. Compounds **1** and **2** possess antiproliferative activity, in particular, tambjamine K (**1**) displayed high cytotoxicity against both tumor and non-tumor mammalian cells.

Tambjamines are a group of alkaloids that have been isolated from bacteria and marine invertebrates including bryozoans, nudibranchs and ascidians.[1–5] From a structural point of view, tambjamines belong to the group of 4-methoxypyrrolic natural products, which includes several antitumor compounds such as those belonging to the tripyrrolic prodigiosin family.[6] The structure of tambjamines is characterized by a piyrrole ring displaying a second pyrrole system at C-2, an enamine moiety at C-5, and a methoxy group at C-4. In the majority of these compounds the enamine nitrogen is substituted with a short saturated alkyl chain.

Tambjamines seem to be implicated in the chemical defense mechanisms of the organisms from which they are obtained.[7,8] Besides their ecological role, a number of these alkaloids possess a wide spectrum of pharmacological properties including antitumor, antimicrobial and immunosuppressive activities.[2,9,10] In particular, the ability to intercalate DNA and facilitate single-strand DNA oxidative cleavage has been demonstrated for both tambjamines E[8] and D.[10]

In the course of our study on the secondary metabolites of shelless mollusks[11,12] that, in the last years, led us to discover the antitumor jorumycin,[13,14] we investigated the chemical content of the nudibranch *Tambja ceutae* García-Gómez and Ortea, 1988, collected off the Azores (Atlantic Ocean). Its prey, the bryozoan *Bugula dentata*, was also analyzed. We now report the characterization of a new tambjamine, tambjamine K (**1**),[15] isolated together with the known related metabolites, tetrapyrrole **2**[16] and tambjamines A (**3**)[1] and B (**4**)[1] (Fig. 1).

Specimens of both the nudibranch *T. ceutae* (15 individuals, size from 27 to 90 mm in length) and the bryozoan *B. dentata* (dry weight 8.5 g) were collected from the port of Horta (Faial island, Azores) by SCUBA diving during August 2007. The material was immediately frozen at −20 °C and transferred to ICB (Pozzuoli, Naples) for the chemical study. The mollusk and the bryozoan were separately extracted with acetone. The ethereal soluble portions of both acetone extracts were compared by TLC and subsequently fractionated by LH-20 Sephadex column and SiO_2 preparative TLC chromatography.[17,18] Tambjamine K (**1**) was the main component of *T. ceutae* whereas it was present only in a very small amount in the extract of *B. dentata*, the main metabolite of which was tetrapyrrole **2**.[16] Minor tambjamines A (**3**)[1] and B (**4**)[1] were isolated as a mixture from the extract of the mollusk. The known molecules **2**–**4** were identified by comparison of NMR and mass data with the literature.[1,16]

Tambjamine K (**1**) had the molecular formula $C_{15}H_{21}N_3O$ as deduced by HRESIMS on the molecular peak at *m/z* 260.1758 $[M+H]^+$. Analysis of the NMR spectra of **1** (Table 1) revealed the presence of

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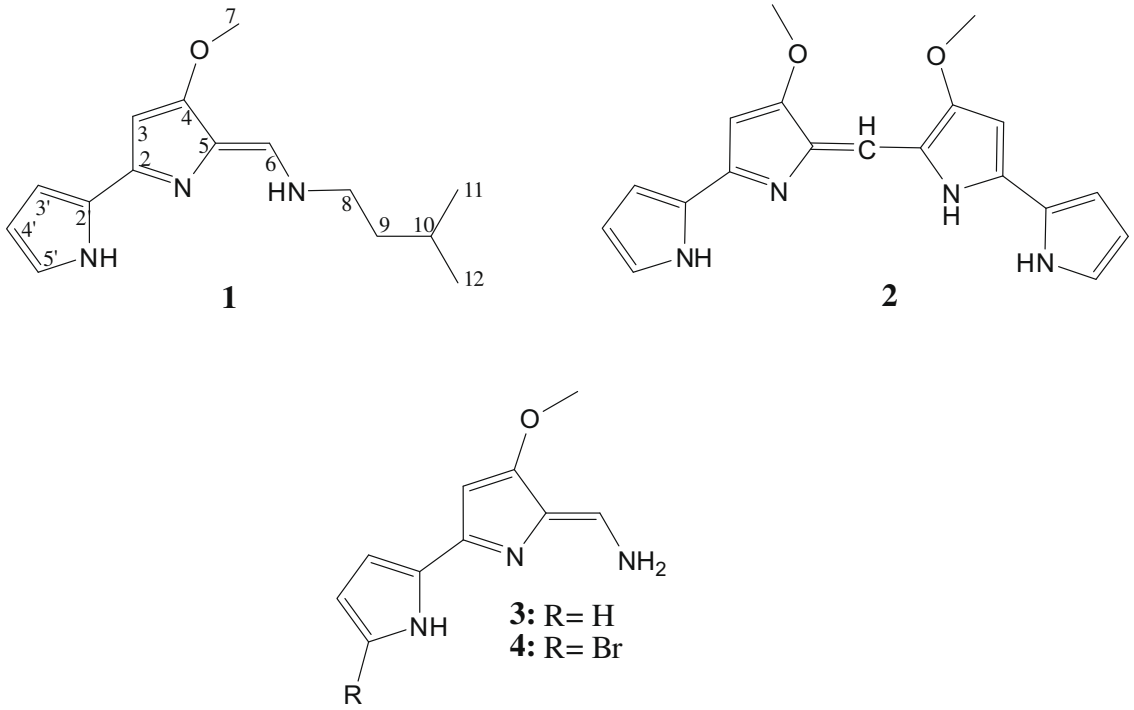

Figure 1. Bromopyrrole alkaloids isolated from the mollusk *T. ceutae* and the bryozoan *B. dentata*.

Table 1
NMR spectral data[a,b] of tambjamine K (**1**)

C	δ_H(mult, *J* in Hz)	δ_C	m	HMBC
2	—	143.8	s	H-3
3	6.00, s	91.5	d	—
4	—	164.1	s	H_3-7
5	—	110.8	s	H-3, H-6
6	7.36, d (15.0)	140.1	d	H_2-8, H-6
7	3.95, s	58.4	q	-
8	3.49, m	49.4	t	H-6, H_2-9
9	1.64, m	38.9	t	H_2-8, H_3-11, H_3-12
10	1.70, m	25.5	d	H_2-8, H_2-9
11	0.96, d (6.4)	22.3	q	H_2-8, H_2-9
12	0.96, d (6.4)	22.3	q	H_2-8, H_2-9
NH	9.23,10.70, 12.50	—	—	—
2′	—	122.3	s	H-5′, H-4′, H-3′
3′	6.77, m	113.7	d	H-5′
4′	6.30, m	110.7	d	H-5′
5′	7.07, m	124.5	d	H-4″, H-3′

[a] The spectra were recorded in $CDCl_3$ at 300 MHz and 400 MHz.
[b] Assignments made by ^1H–^1H COSY, HSQC and HMBC (*J* = 10 Hz) experiments.

structural features characteristic of the alkaloids belonging to the tambjamine class. The ^1H NMR spectrum contained four methine signals at δ 7.07 (m, H-5′), 6.77 (m, H-3′), 6.30 (m, H-4′), and 6.00 (s, H-3) and a 3H singlet at δ 3.95 (H_3-7) that were consistent with the 4-methoxy-2,2′-bis-pyrrole moiety the same as tambjamine A (**3**).[1] The enamine fragment at C-5 was evidenced by the presence of a methine doublet at δ 7.36 (1H, d, *J* = 15.0 Hz, H-6) in the proton spectrum and signals at δ 110.8 (s, C-5) and 140.1 (d, C-6) in the carbon spectrum. Analysis of the ^1H–^1H COSY experiment of **1** showed a spin system [3.49 (2H, m, H_2-8), 1.64 (2H, m, H_2-9), 1.70 (1H, m, H-10), 0.96 (6H, s, H_3-11 and H_3-12) due to an isopentenyl residue, which was suggested to be linked to the enamine nitrogen. Thus, tambjamine K was the isopentenyl derivative of the co-occurring tambjamine A (**3**).[1] The complete proton and carbon assignments of tambjamine K (**1**) was achieved by 2D NMR experiments (^1H–^1H COSY, HSQC, and HMBC) (Table 1).

The significant cytotoxic activity showed by several members of tambjamine family, probably related to their DNA-targeting properties, prompted us to investigate the bioactivity of tambjamine K (**1**) and tetrapyrrole **2** on tumor and non-tumor cell lines by evaluation of cell growth and viability. CaCo-2 human epithelial colorectal adenocarcinoma cells, HeLa human cervical cancer cells, C6 rat glioma cells, H9c2 rat cardiac myoblast cells and 3T3-L1 murine fibroblasts were treated for 48 h with various concentrations of compounds **1** and **2**. Then, the cells were counted and the MTT assay was performed in order to evaluate cell viability and proliferation by measuring the level of mitochondrial dehydrogenase activity.[19] The results showed that both tambjamine K (**1**) and tetrapyrrole **2** exhibit a remarkable and concentration-dependent cytotoxic activity against both tumor and non-tumor mammalian cells. The IC_{50} values calculated from the obtained data are reported in Table 2. As expected, tambjamine K (**1**) displayed an high antiproliferative effect against all tested cell lines. In particular, **1** showed an IC_{50} value against tumor CaCo-2 cells within the nanomolar range (3.5 nM), which is generally considered as a strong

Table 2
Cell growth inhibition

Compound	IC_{50}, μM[a] (CaCo-2 cells)	IC_{50}[a], μM (HeLa cells)	IC_{50}[a], μM (C6 cells)	IC_{50}[a], μM (H9c2 cells)	IC_{50}[a], μM (3T3-L1 cells)
1	$3.5 \times 10^{-3} \pm 1.4 \times 10^{-3}$	14.6 ± 9	14 ± 5.4	2.7 ± 2	19 ± 12
2	91 ± 15	70 ± 18	7 ± 4.2	107 ± 8	24 ± 8.5

[a] IC_{50} values are expressed as mean ± SEM (*n* = 24) of four independent experiments.

cytotoxic activity. Interestingly, tetrapyrrole **2** resulted more active than tambjamine K (**1**) against C6 glioma cells.

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18. An aliquot (206 mg) of the Et_2O extract (1.5 g) of *B. dentata* was fractionated by LH-20 Sephadex column ($CHCl_3$/MeOH, 1:1) to give a fraction (25 mg) containing **1** and a main fraction (72 mg) constituted by crude **2**. An aliquot (18 mg) of the latter fraction was subsequently purified by SiO_2–TLC plate ($CHCl_3$/MeOH, 9:1) to give 15.0 mg of pure **2**.
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