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Cystodytin L, a Pyridoacridine Alkaloid from the Senegalese Tunicate *Cystodytes sp*.

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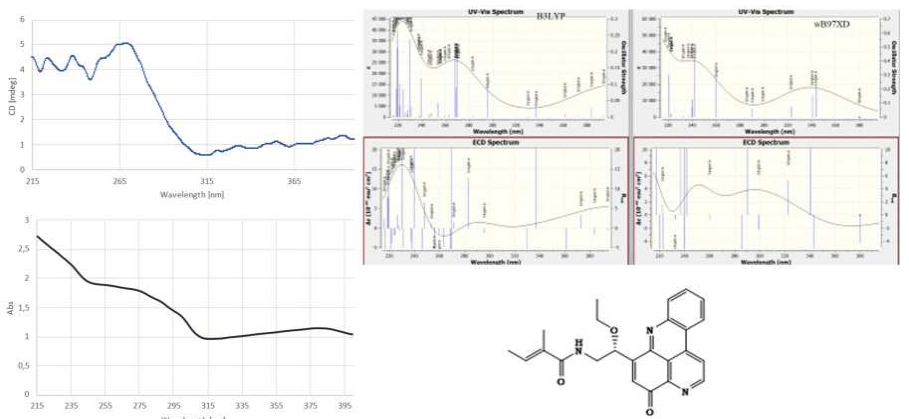
Abstract

Chemical investigation of the brown-colored tunicate *Cystodytes sp.* yielded six pyridoacridine alkaloids, including the known metabolites arnoamine C (**1**), styelsamine C (**2**), cystodytin B (**3**), E (**4**) and G (**5**) and the new compound, cystodytin L (**6**).

Their chemical structures were established on the basis of combination of $[\alpha]_D$, physical and spectroscopic methods 1D and 2D NMR, in addition to mass spectrometry and comparison with literature data. The absolute configuration of C-12 in compounds 4-6 was identified as *R* by electronic circular dichroism (ECD) spectroscopy coupled with time-dependent density functional theory calculations.



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Keywords and phrases: tunicate; *Cystodytes sp*.; cystodytin; pyridoacridine alkaloids; marine metabolites.

1. Introduction

Marine natural products chemistry was conceived in 1951 when Bergmann and Feeny reported on the isolation of the unusual nucleosides spongouridin and spongothymidin from the sponge *Cryptotethya crypta*, which served as lead structures for antiviral drugs such as Ara-A [1]. More than decade later, they continue to be in the spotlight in the global drug discovery endeavour. Currently, more than 30,000 structurally diverse secondary metabolites from marine sources have been isolated, making marine natural products a profound, renewable source to investigated novel drug compound [2].

Senegal's 700-kilometre-long coastline connect the Saint-Louis region to the natural region of Casamance from North to South. It is populated by easily accessible, abundant and varied flora and fauna. The study of the secondary metabolites from the species of these flora and fauna is particular importance for Senegal given the potential economic and medical impact.

As part of our continuing investigation of bioactive natural products from Senegalese marine microorganisms, we report here a new pyridoacridine alkaloid of brown-colored tunicate *Cystodytes sp.* Pyridoacridines are the largest group of alkaloids isolated only from marine organisms. They have been reported from sponges [3,4], ascidians [5,6], anemones [7], tunicates [8,9], and prosobranch mollusk [10]. They are characterised by 11*H*-pyrido[4,3,2-mn]acridine moiety, and are highly colored due their highly conjugated system. Almost all natural pyridoacridines have been reported to possess significant cytotoxicity due to a highly planar electron-deficient aromatic ring system that can intercalate DNA, resulting in the inhibition of cell growth [11,12]. Pyridoacridines also have certain specific biological properties in living systems, including antibacterial [13], antifungal [14], antiviral [15], anti-HIV [16], antiparasitic [17], and topoisomerase II inhibitors [18]. They represent a source of new lead structures for the development of future generation of therapeutic agents.

2. Experimental

2.1. *General procedures*

All the organic solvents used for material extraction were of analytical grade and purchased from Sigma Aldrich (France).

Optical rotation measurements were conducted on an Anton Paar® MCP-100

polarimeter at 589 nm (l = 0.5 dm, 20°C). Circular dichroism experiments were recorded at 20°C on a Jasco J-180 spectropolarimeter. High-resolution mass spectra (HRMS) were recorded with an Thermo LCQ Advantage. NMR spectra were obtained in DMSO on a 400 and 500 MHz Bruker Avance. NMR chemical shifts were expressed in parts per million (ppm) referenced to residual DMSO solvent signals (δ_H 2.50 for ^{1}H and δ_C 39.52 for ^{13}C) and $CDCl_3$ solvent signals (δ_H 7.26 for ^{1}H and δ_C 77.0 for ^{13}C).

HPLC-PDA-ELSD analyses were performed with a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, MA) coupled with a Waters 996 photodiode array detector and a Sedex 55 evaporative light-scattering detector (SEDERE, France), using a bifunctional Macherey-Nagel NUCLEODUR® Sphinx RP column (250 x 4.6 mm, 5 µm) consisting of a balanced ratio of propylphenyl and C18 ligands. The mobile phase was composed of H_2O (plus 0.1% formic acid) and acetonitrile (MeCN plus 0.1% formic acid) and the following gradient was used: H_2O:MeCN 90:10 for 5 min, H_2O:MeCN 90:10 to 0:100 for 30 min, 0:100 for 5 min, 0:100 to 90:10 for 15 min (flow: 1.0 mL.min^{-1}, injection volume: 20 µL). Chromatograms were extracted at the following detection wavelengths for visual inspection: 214, 254, and 280 nm.

A part of specimen (5 g) was extracted three times during 10 min with 10 mL of a mixture of MeOH/CH_2Cl_2 (1:1, v/v) in an ultrasonic bath. The filtrates of each extraction were combined, mixed with 1 g of RP C18 silica gel and evaporated to dryness. The latter were then located on to C18 solid phase extraction (SPE) cartridges beforehand conditioned (2 g, Phenomenex Strata). The SPE column was first washed with 10 mL of H_2O for desalting and then eluted with 10 mL of MeOH/CH_2Cl_2 (1:1, v/v) in a 20 mL volumetric flask. The organic phase, obtained after concentration under reduced pressure, was used for HPLC-PDA-ELSD analyses.

2.2. *Animal material*

The brown-colored tunicate *Cystodytes sp.* Was collected by scuba diving at a depth of 6 m from Rufisque, Senegal in 2019. Freshly collected tunicate was frozen immediately after collection.

2.3. *Extraction and isolation*

The tunicate (504 g) was extracted several times with EtOH (300 mL x 3) followed by a mixture of 1:1 CH_2Cl_2:MeOH (200mL x 2) to yield 19.33 g and 0.50 g respectively of the ethanolic and MeOH:CH_2Cl_2 crude extracts after concentration under reduced pressure. The ethanolic crude extract was fractionated by RP-C18 flash chromatography

(elution with a decreasing polarity gradient of H_2O:MeOH from 1:0 to 0:1, then CH_2Cl_2:MeOH from 1:0 to 0:1). The H_2O:MeOH (1:3, v/v) fraction (119 mg) and MeOH fraction (363.8 mg) were then subjected to a semi-preparative HPLC-DAD (Macherey-Nagel NUCLEODUR® Sphinx RP column, 250 x 10 mm id, 5 µm) using H_2O:MeCN gradient to afford respectively cystodytin B (1.9 mg), cystodytin G (1.9 mg) and, arnoamine C (6.8 mg), cystodytin E (4.4 mg).

The CH_2Cl_2:MeOH crude extract was dissolved in MeOH and adsorbed on a Diaion HP-20 column (Supleco) and eluted with an MeOH/H_2O system. The MeOH fraction (208 mg) was subjected to a semi-preparative HPLC-DAD (Macherey-Nagel NUCLEODUR® Sphinx RP column, 250 x 10 mm id, 5 µm) using H_2O:MeOH gradient to give arnoamine C (1.6 mg), styelsamine C (1.8 mg) cystodytin B (4.8 mg), cystodytin E (1.6 mg) and cystodytin L (2.0 mg).

Arnoamine C (1): orange oil. ^{1}H NMR (400 MHz, DMSO-d_6); δ 10.63 (s, 1H), 9.08 (d, 5.8 Hz, 1H), 9.00 (d, 8.1 Hz, 1H), 8.68 (d, 5.7 Hz, 1H), 8.55 (d, 8.6 Hz, 1H) 8.00 (t, 8.2 Hz, 1H), 7.95 (s, 1H), 7.72 (t, 7.5 Hz, 1H), 7.24 (s, 1H), 6.84 (q, 6.9 Hz, 1H), 1.97 (s, 3H), 1.91 (d, 6.9 Hz, 3H); (+)HRESIMS m/z 356.1373 (calcd for $C_{22}H1_8N_3O_2$ 356.1394, Δmmu 2.1 ppm).

Styelsamine C (2): orange solid. ^{1}H NMR (400 MHz, DMSO-d_6,); δ 12.11 (brs, 1H), 9.95 (brs, 1H), 8.85 (brs, 1H), 8.34 (brs, 1H), 7.95 (brs, 1H), 7.61 (m, 1H), 7.61 (m, 1H), 7.37 (br s, 1H), 7.29 (brs, 1H); (+)ESIMS m/z 263.6).

Cystodytin B (3): yellow cristal. ^{1}H NMR (400 MHz, $CDCl_3$); δ 9.23 (d, 5.4 Hz, 1H), 8.62-8.52 (m, 2H), 8.30 (d, 8.0 Hz, 1H), 7.93 (t, 7.6 Hz, 1H), 7.84 (t, 7.7 Hz, 1H), 6.93 (s, 1H), 6.38-6.24 (m, 2H), 3.82 (q, 6.0 Hz, 2H), 3.34 (t, 6.4 Hz, 2H), 1.74 (s, 3H), 1.65 (d, 7.7 Hz, 3H); (+)HRESIMS m/z 358.1547 (calcd for $C_{22}H_{20}N_3O_2$ 356.1550, Δmmu0.3 ppm).

Cystodytin E (4): Brown-colored amorphous solid; $[\alpha]^{20}_D$-181.8 (c 0.05, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6,); δ 9.25 (d, 5.2 Hz, 1H), 9.05 (d, 5.6 Hz, 1H), 8.95 (d, 7.8 Hz, 1H), 8.23 (d, 8.1 Hz, 1H), 8.01 (t, 7.4 Hz, 1H) 7.94 (d, 7.1 Hz, 1H), 7.72 (t, 6.1 Hz, 1H), 6.97 (s, 1H), 6.12-6.08 (m, 1H), 5.62 (s, 1H), 3.75 (t, 5.6 Hz, 1H), 3.63 (dd, 13.1, 5.3 Hz, 1H), 1.56-1.55 (m, 6H); (+)HRESIMS m/z 374.1483 (calcd for $C_{22}H_{20}N_3O_3$ 374.1499, Δmmu1.6 ppm).

Cystodytin G (5): Brown-colored amorphous solid; $[\alpha]^{20}_D$-133.3 (c 0.05, EtOH); ^{1}H NMR (500 MHz, DMSO-d_6); δ 9.26 (m, 1H), 9.06 (m, 1H), 8.96 (m, 1H), 8.23

(d, 7.9 Hz, 1H), 8.02 (m, 1H), 7.94 (m, 1H), 7.75 (t, 6.2 Hz, 1H), 6.81 (s, 1H), 6.07 (m, 1H), 5.33 (t, 4.6 Hz, 1H), 3.79-3.62 (m, 2H), 3.42 (s, 3H), 1.56-1.50 (m, 6H); (+)HRESIMS m/z 388.1649 (calcd for $C_{23}H_{22}N_3O_3$ 388.1656, Δmmu0.7 ppm).

Cystodytin L (6): Brown-colored amorphous solid; $[\alpha]^{20}_D$ - 120 (c 0.05, EtOH); NMR data see Table 1; (+)HREIMS m/z 402.1794 (calcd for $C_{24}H_{24}N_3O_3$ 402.1812, Δmmu1.8 ppm).

3. Results and Discussion

Compound (**6**) (Figure 1), a chiral levorotatory compound, was obtained as a brown-colored amorphous solid. Its molecular formula was determined as $C_{24}H_{23}N_3O_3$ by (+)HRESIMS (m/z 402.1794, $[M + H]^+$) (Figure 2). Its structure was elucidated by interpretation of MS and NMR data (Figures 3-7) and comparison to spectral data of cystodytins [19-21]. The molecular mass of cystodytin L (**6**) is increase of 44 mass units compared with cystodytin B (**3**), suggested one more ethoxy group.

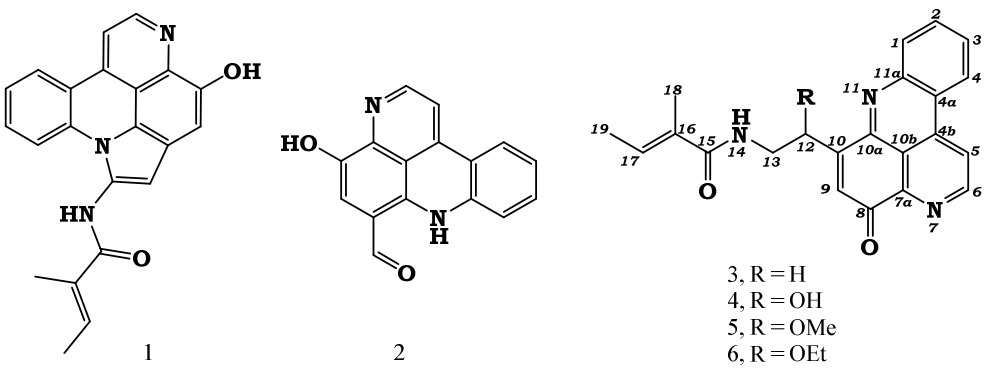


Figure 1. Chemical structures of the pyridoacridine alkaloids isolated from the brown-colored tunicate *Cystodytes sp.*

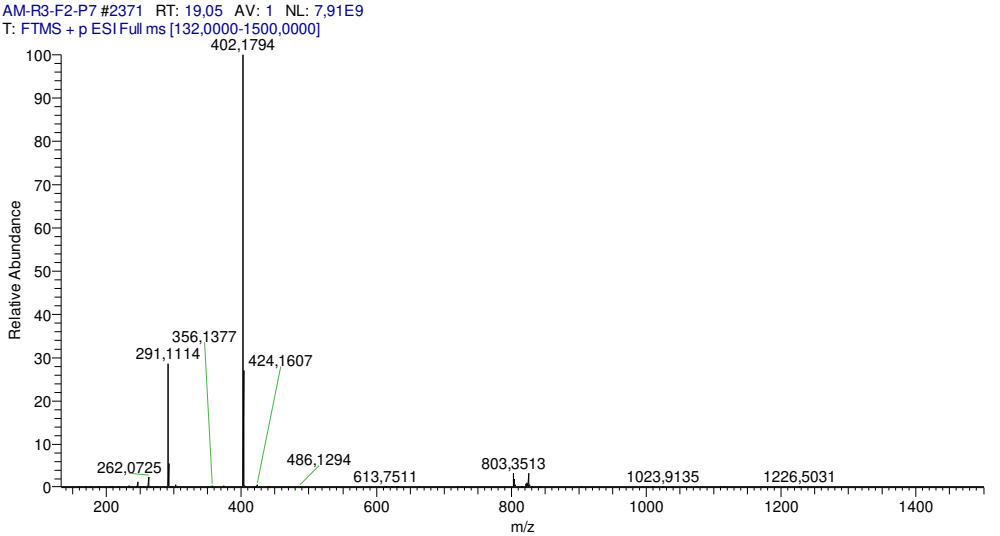


Figure 2. (+)HRESIMS spectrum of cystodytin L.

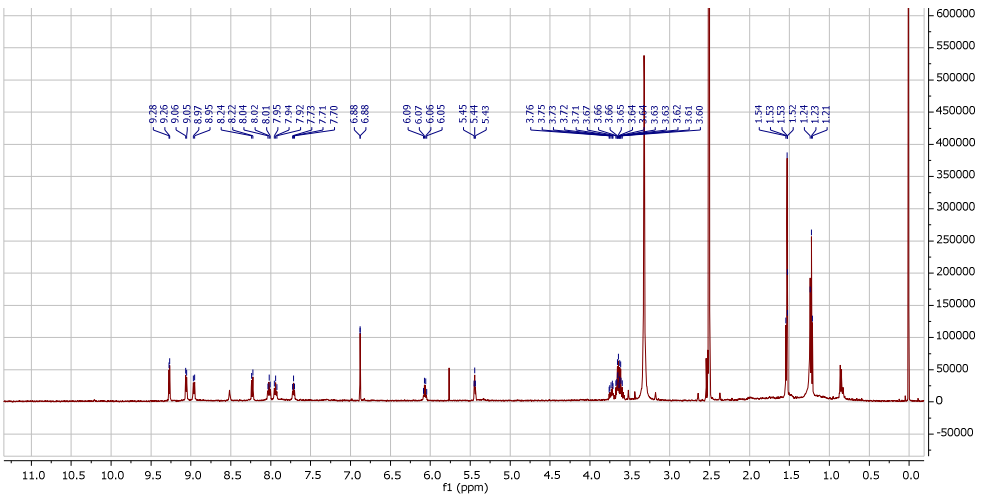


Figure 3. ^{1}H NMR spectrum of cystodytin L.

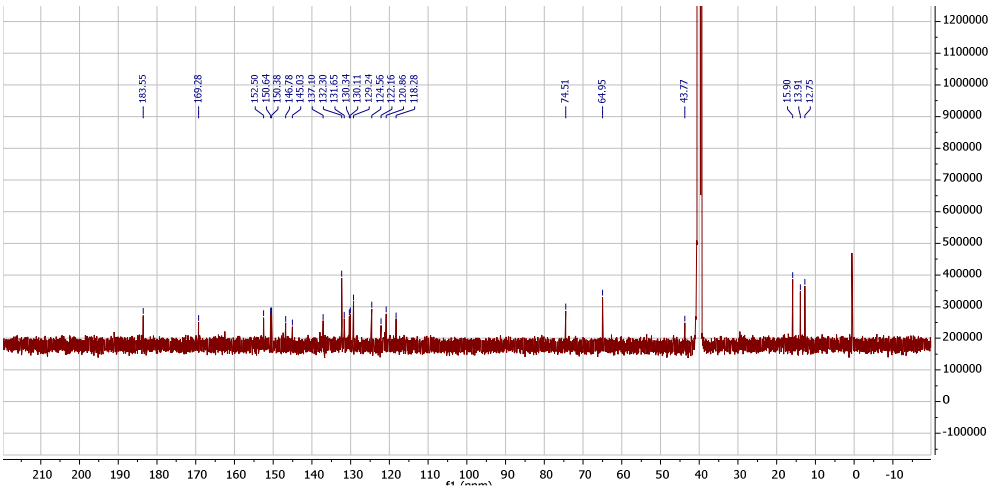


Figure 4. ^{13}C NMR spectrum of cystodytin L.

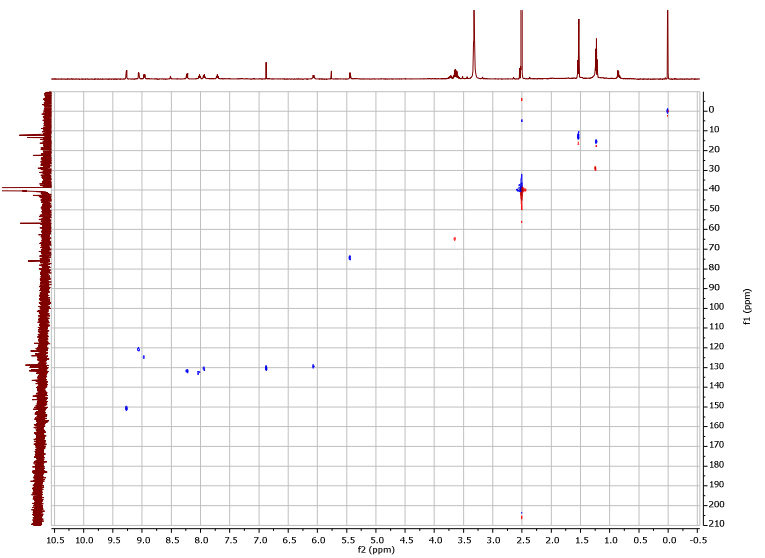
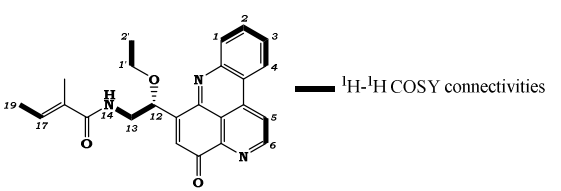


Figure 5. HSQC spectrum of cystodytin L.



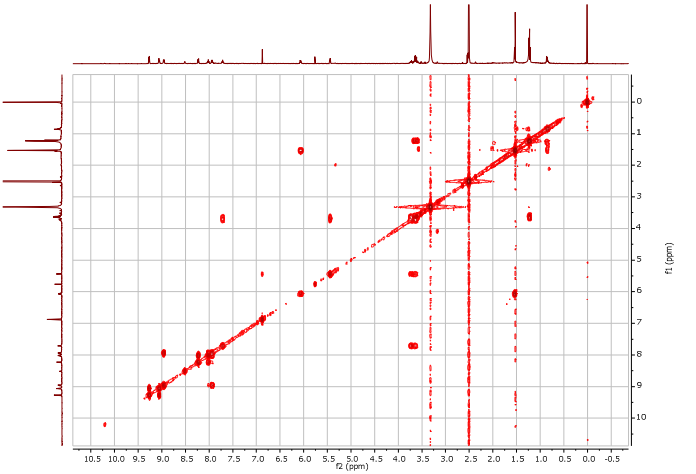


Figure 6. ^{1}H-^{1}H COSY spectrum of cystodytin L (**6**).

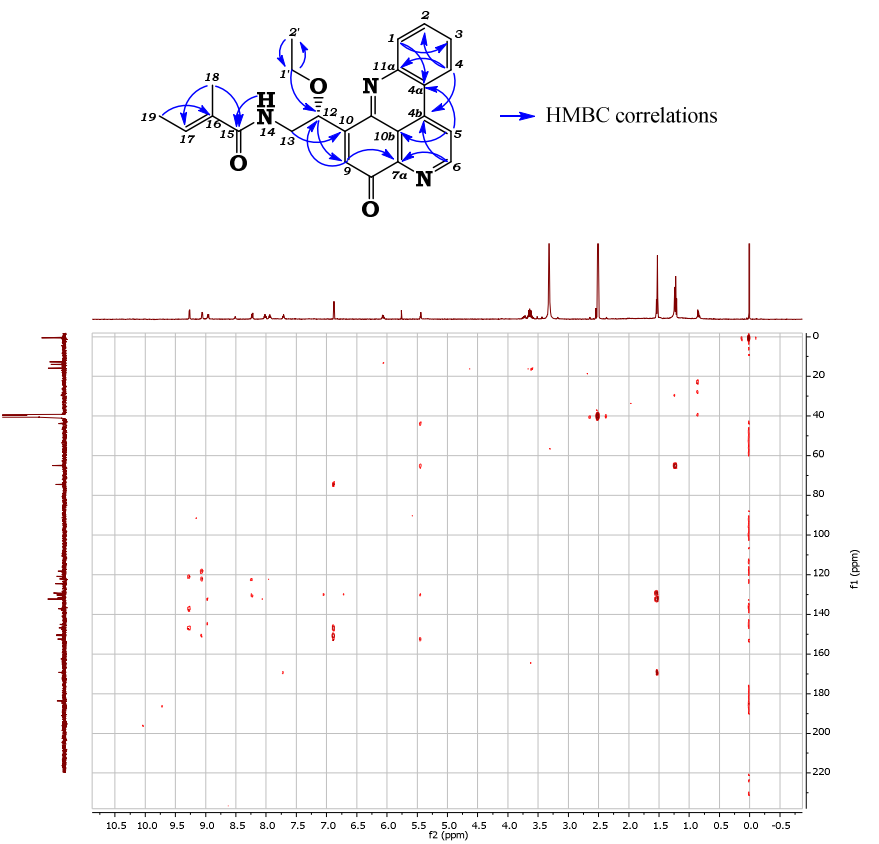


Figure 7. HMBC spectrum of cystodytin L.

The ^{1}H-^{1}H COSY, HSQC and HMBC experiments in DMSO-d_6 (Table) indicated for **6** a pyridoacridine skeleton fused with the ethylamine chain as a cystodytins B (**3**). This hypothesis was supported by close similarities NMR data of **6** and cystodytin B [20]. The main differences between **6** and **3** were located on C-12 position of the ethylamine chain. This is confirmed by the presence of a spin system consisting of the signal at δ_H 3.64 (H-1', m) and δ_H 1.22 (H-2', t) by inspection of the ^{1}H-^{1}H COSY (Figure 5), consisting by a ethoxy group. The HMBC correlation between H-1' and C-12 (δ_C 74.51) (Figure 7) confirmed the 12-O-1' connexion between the ethoxy group and the ethylamine chain.

Table. NMR data (500/125 MHz, DMSO-d_6) for cystodytin L (6).

N°	δ_C (ppm)/	mult.	δ_H (ppm)/*J* (Hz)/ mult.			COSY	HMBC
1	131.65	CH	8.23	8.1	d	2	
2	132.30	CH	8.02	7.6	t	1	4
3	130.34	CH	7.94	7.6	t	2, 4	1
4	124.56	CH	8.96	8.1	d	3	
4a	122.16	qC					1, 5
4b	137.10	qC					4, 6
5	120.86	CH	9.06	5.5	d	6	6
6	150.64	CH	9.27	5.5	d	5	5
7a	146.78	qC					6, 9
8	183.55	qC					
9	130.11	CH	6.88		s		12
10	152.50	qC					12, 13
10a	150.38	qC					9
10b	118.28	qC					5
11a	145.03	qC					4
12	74.51	CH	5.44	4.6	t	13	9
13	43.77	CH_2	3.73		m	12, 14	12
			3.64		m	12, 14	12
14		NH	7.71	6.2	t	13	
15	169.28	qC					14, 18
16	132.30	qC					19
17	129.24	CH	6.07	6.9	q	19	18
18	12.75	CH_3	1.53		m		
19	13.91	CH_3	1,53		m	17	17
1'	64.95	CH_2	3.64		m	2'	12, 2'
2'	15.90	CH_3	1,22	7.2	t	1'	1'

4. Conclusion

In conclusion, our search for marine-derived bioactive compounds has led to the investigation of specimen of the Senegalese marine brown-colored tunicate *Cystodytes sp*. One new pyridoacridine alkaloid, cystodytin L (6) and the known compounds arnoamine C (**1**), styelsamine C (**2**), cystodytin B (**3**), E (**4**) and G (**5**) were isolated. Their chemical structures were assigned using combination of $[\alpha]_D$, physical and spectroscopic methods 1D and 2D NMR, in addition to mass spectrometry and comparison with literature data.

Pyridoacridine alkaloids are of high importance in medicinal chemistry and commonly found in many compounds of practical importance, ranging from natural compounds to pharmaceutical agents. Now, it can be hope that continuing interest in structure modulation of these marine metabolites may provide novel potential medicinal agents.

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