Earthline Journal of Chemical Sciences
E-ISSN: 2581-9003; CODEN: EJCSB4
Volume 12, Number 3, 2025, Pages 359-366
https://doi.org/10.34198/ejcs.12325.359366

Dihydrocystodytine G, a new tetracyclic aromatic alkaloid isolated from a specimen of the marine tunicate *Cystodytes* sp. collected off Rufisque - Senegal

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Abstract

Our recent research on the brown tunic of *Cystodytes* sp. has led to the isolation of four tetracyclic aromatic alkaloids. Among these compounds, cystodytin B (1) and cystodytin G (2) have already been well characterized. Cystodytin L (3) was also found, a compound we had previously identified and published. Above all, the study highlighted the discovery of a new alkaloid: Dihydrocystodytine G (4).

The chemical structures of these compounds were elucidated by combining the determination of specific rotatory power ([α]D), various spectroscopic techniques (1D and 2D NMR), mass spectrometry and comparison with literature data. In addition, the absolute C-12 configuration of compounds 2 to 4 was established as R using electron circular dichroism (ECD) spectroscopy and time-dependent DFT (Density Functional Theory) calculations.

In this article, we describe the isolation and structural elucidation of this new compound.

1. Introduction

Tetracyclic alkaloids constitute an important class of marine natural products, standing out as the largest group of alkaloids isolated exclusively from marine organisms [1]. Their presence has been reported in various species such as sponges [2,3] and ascidians [4,5].

Pyridoacridines are characterized by a common 11H-pyrido[4,3,2-mn]acridine backbone. This highly planar, richly conjugated tetracyclic motif is responsible for their strong coloration and distinctive

Received: July 14, 2025; Accepted: August 12, 2025; Published: August 21, 2025

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

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physicochemical properties [6]. The unique structure of pyridoacridines also confers on them a wide range of significant biological activities: Cytotoxicity, Anticancer [7], Antimicrobial [8], Antiviral and Antiparasitic [9,10] activities, making them promising candidates for drug discovery.

As part of our research into bioactive secondary metabolites from Senegalese marine species, we report the isolation of a new pyridoacridine compound, dihydrocystodytin G (4), from the brown tunic of *Cystodytes* sp. (collected off the coast of Senegal). This study also made it possible to re-isolate several pyridoacridines already known from this or related species, notably cystodytin B (1), cystodytin G (2), and cystodytin L (3), the latter having been the subject of a previous publication by our group [11].

2. Experimental Part

2.1. General procedures

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

For the analyses, optical rotation measurements were performed on an Anton Paar® MCP-100 polarimeter, using a wavelength of 589 nm and a temperature of 20°C (l = 0.5 dm). Circular dichroism was recorded at 20°C using a Jasco J-180 spectropolarimeter. High-resolution mass spectra (HRMS) were obtained using a Thermo LCQ Advantage system. Finally, nuclear magnetic resonance (NMR) spectra were acquired on Bruker Avance instruments operating at 400 and 500 MHz, using DMSO as solvent. NMR chemical shift values are expressed in parts per million (ppm) and were calibrated against the signals of the residual solvents: DMSO (δ_H 2.50 for ^{1}H and δ_C 39.52 for ^{13}C) and CDCl3 (δ_H 7.26 for ^{1}H and δ_C 77.0 for ^{13}C).

In order to characterize extract profiles, high-performance liquid chromatography (HPLC) analyses, coupled with photodiode array (PDA) detection and an evaporative light scattering (ELSD) detector, were implemented. The set-up included a Waters Alliance 2695 system (Waters Corporation, Milford, MA), a Waters 996 PDA detector and a Sedex 55 ELSD detector (SEDERE, France). Analyte separation was optimized on a Macherey-Nagel NUCLEODUR® Sphinx RP column (250 x 4.6 mm, 5 µm), equipped with a bifunctional stationary phase (propylphenyl and C18).

Elution was carried out with a mobile phase composed of water (containing 0.1% formic acid) and acetonitrile (containing 0.1% formic acid), using the following gradient: the acetonitrile proportion was increased from 10% (for 5 min) to 100% (in 30 min), then maintained at 100% (5 min), before returning to 10% (in 15 min). The flow rate was maintained at 1.0 mL.min-¹ and the injection volume at 20 µL. Chromatograms were inspected visually at wavelengths of 214, 254 and 280 nm.

A 4 g sample was subjected to triple sonication extraction (10 min/extraction) using 10 mL MeOH/CH_2Cl_2 (1:1, v/v). The combined extracts were adsorbed onto 1 g of C_{18} RP silica gel, then evaporated to dryness. The resulting material was purified using a pre-conditioned SPE C_{18} cartridge (2 g, Phenomenex Strata). The column was washed with 10 mL H_2O for desalting, then eluted with 10 mL MeOH/CH_2Cl_2 (1:1, v/v) in a 20 mL flask. After concentration in vacuo, the organic phase was used directly for HPLC-PDA-ELSD analysis.

2.2. Biological material

The biological specimen, an ascidian of the species *Cystodytes* sp., recognizable by its brown coloration, was collected in 2019 by scuba diving at a depth of 6 meters, off the coast of Rufisque, Senegal. As soon as it was collected, the ascidian was immediately frozen to preserve its integrity.

2.3. Extraction and isolation procedure

Ascidia (504 g) was first subjected to repeated extraction with ethanol (3 x 300 mL), followed by extraction with a 1 :1 mixture of CH_2Cl_2 : MeOH (2 x 200 mL). These steps yielded two crude extracts after concentration under reduced pressure: a 19.33 g ethanolic extract and a 0.50 g CH_2Cl_2 : MeOH extract.

The crude ethanolic extract was then purified by RP-C18 flash chromatography. Elution was carried out using a gradient of decreasing polarity, first with H_2O : MeOH (from 1 : 0 to 0 : 1), then with CH_2Cl_2 : MeOH (from 1 : 0 to 0 : 1). From the fractions obtained, the H_2O : MeOH (1 : 3, v/v) fraction (105 mg) and the MeOH fraction (255.7 mg) were selected. They were then analyzed by semi-preparative HPLC-DAD (Macherey-Nagel NUCLEODURR® Sphinx RP column, 250 x 10 mmdi, 5 µm) using a H2O : MeCN gradient. This purification isolated cystodytin B (1.6 mg), cystodytin G (1.8 mg) and cystodytin L (2.3 mg).

The crude CH_2Cl_2: MeOH extract was dissolved in MeOH and adsorbed on a Diaion HP-20 column (Supleco), then eluted with a MeOH/H_2O system. The ethanolic fraction (208 mg) was then subjected to semi-preparative HPLC-DAD (Macherey-Nagel NUCLEODURR® Sphinx RP column, 250 x 10 mmdi, 5 µm) with a H_2O : MeOH gradient. This last step was used to isolate cystodytin B (1.6 mg) and dihydrocystodytine G (1.8 mg).

Cystodytin B (1): Yellow crystal. NMR ^{1}H (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 (calculated for $C_{22}H_{20}N_3O_2$ 356.1550, Δmmu 0.3 ppm).

Cystodytin G (2): Brown amorphous solid; [α]D20 -133.3 (c 0.05, EtOH). NMR ^{1}H (500 MHz, DMSO-d6): 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 (calculated for $C_{23}H_{22}N_3O_3$ 388.1656, Δmmu 0.7 ppm).

Cystodytine L (3): Brown amorphous solid; [α]D20 -120 (c 0,05, EtOH). RMN ^{1}H (400 MHz, DMSO-d6,); δ 9.27 (d, 5. 5 Hz, 1H), 9.06 (d, 5.5 Hz, 1H), 8.96 (d, 8.1 Hz, 1H), 8.23 (d, 8.1 Hz, 1H), 8.02 (t, 7.6 Hz, 1H) 7.94 (t, 7.6 Hz, 1H), 7. 71 (t, 6.1 Hz, 1H), 6.88 (s, 1H), 6.07 (q, 1H), 5.44 (t, 4.6, 1H), 3.64-3.73 (m, 2H), 3.63 (dd, 13.1, 5.3 Hz, 1H), 1. 53 (m, 6H); 3.64 (m, 2H), 1.22 (t, 7.2, 6H). (+)HREIMS m/z 402,1794 (calculated for $C_{24}H_{24}N_3O_3$ 402,1812, Δmmu 1,8 ppm).

Dihydrocystodytin G (4): Brown amorphous solid. NMR data are shown in Table 1; (+)HREIMS m/z 390.1809 (calculated for $C_{23}H_{23}N_3O_3$ 390.1912, Δmmu1.8 ppm).

3. Results and Discussion

We obtained compound (4) (Figure 1) as a brown-colored amorphous solid, characterized by its chirality and levorotatory power. Its molecular formula, $C_{23}H_{23}N_3O_3$, was accurately established via (+)HRESIMS (m/z 390.1809, [M + H]+ Δmmu 0.3 ppm), as shown in Figure 2. The structure of this compound was determined by rigorous interpretation of mass spectrometry and NMR data (Figures 3-7), supplemented by confrontation with previously known cystodytin spectral information [12, 13, 14]. A key point is the hydrogenation of the double bond between carbons C-3 and C-4 of cystodytin M (4) compared with cystodytin G (2). This hydrogenation is confirmed by the presence of a spin system composed of six aromatic signals δ_H 9.26; 9.06; 8.96; 8.23; 8.02; 7.94 for cystodytin G (2) versus four at δ_H 9.25; 8.65; 8.13; 7.96 for dihydrocystodytin G (4) (Figure 1).

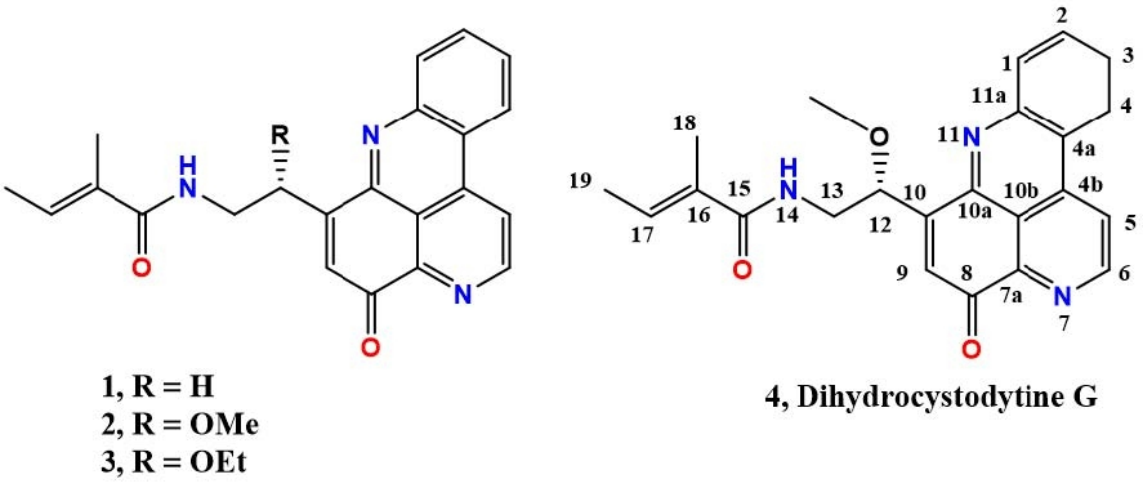

Figure 1. Chemical structures of tetracyclic alkaloids isolated from the brown tunicate *Cystodytes* sp.

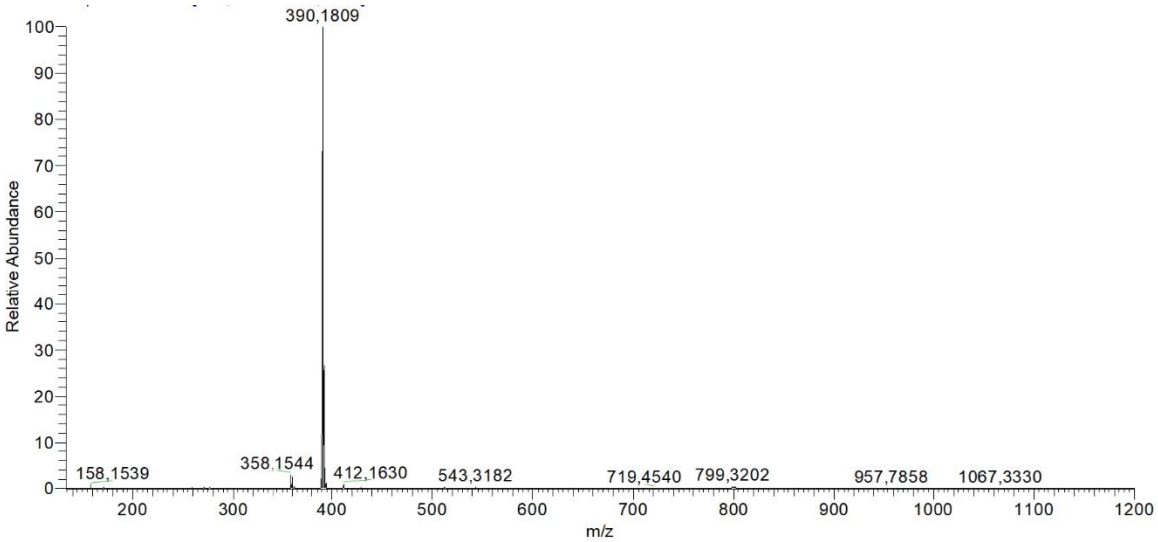

Figure 2. (+)HRESIMS spectrum of dihydrocystodytin G.

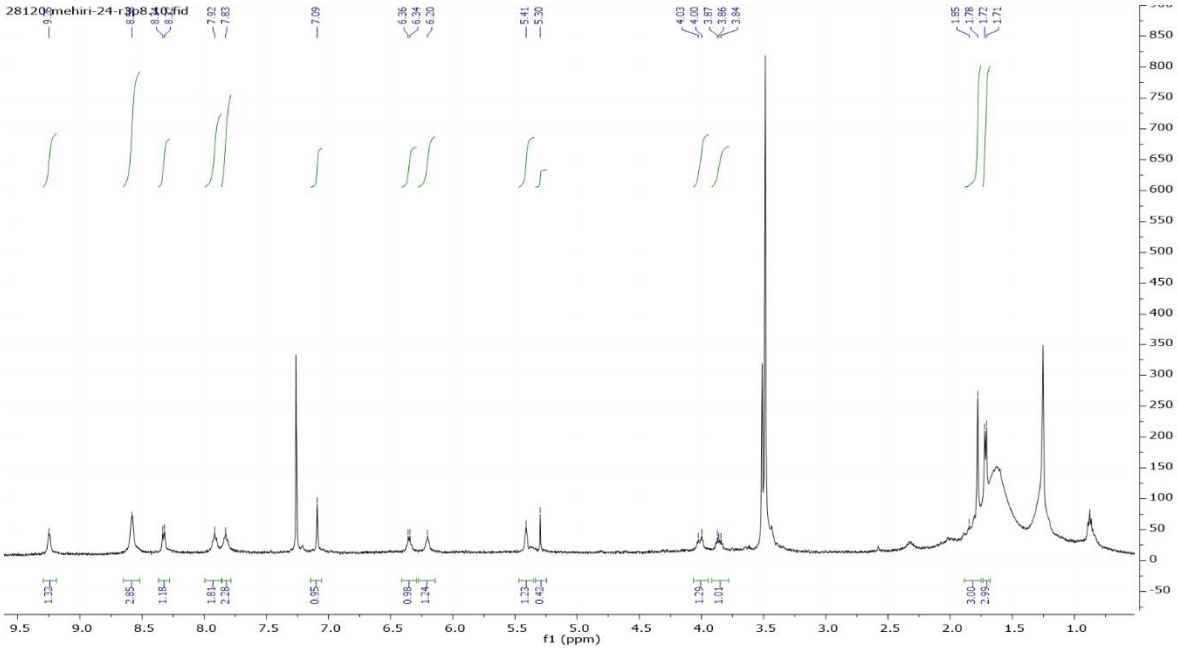

Figure 3. [1]HMR spectrum of dihydrocystodytin G.

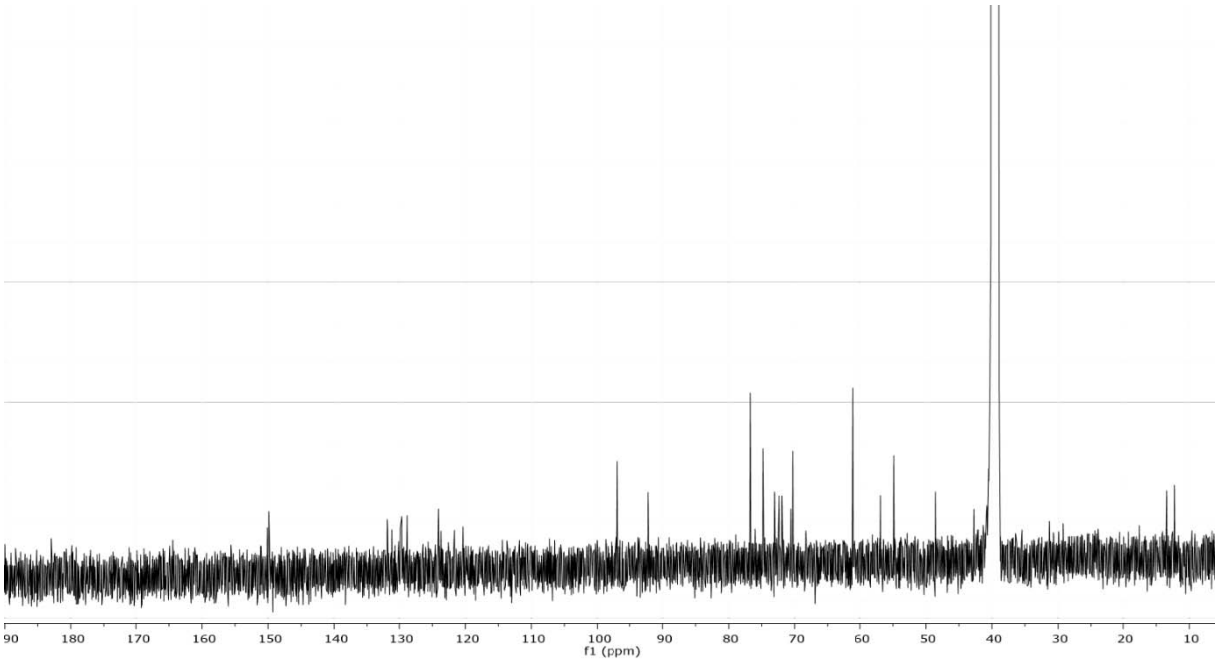

Figure 4. [13]C-NMR spectrum of dihydrocystodytin G.

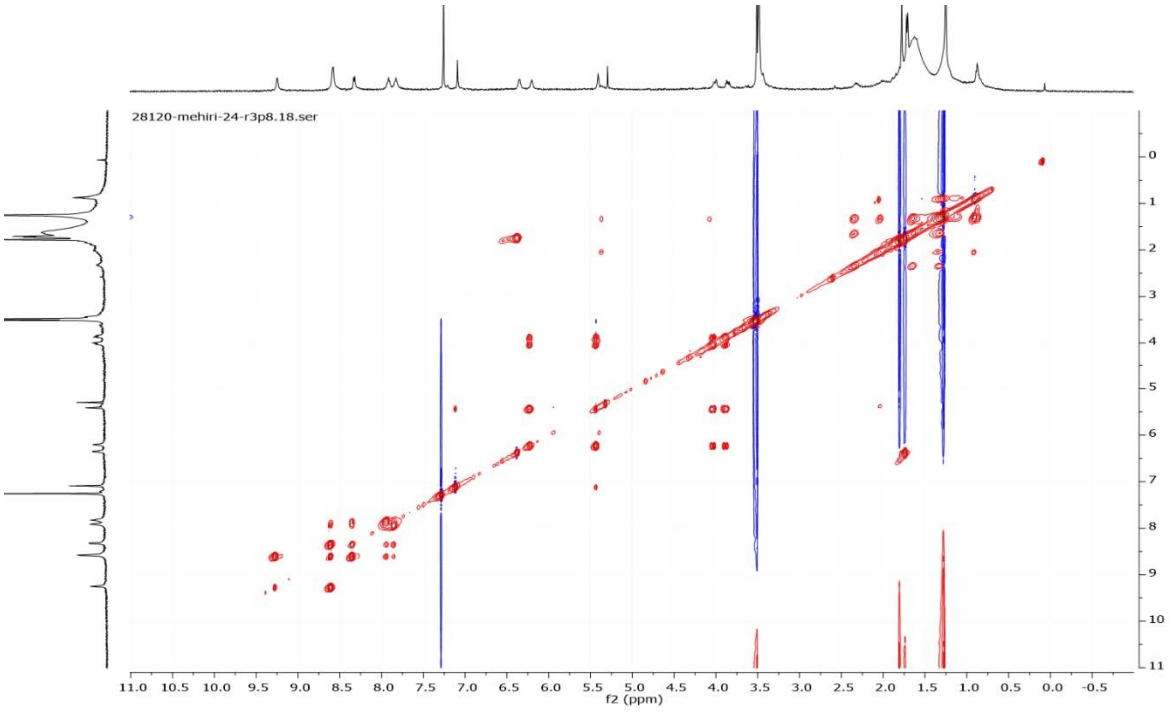

Figure 5. COSY ^{1}H-^{1}H spectrum of dihydrocystodytin G.

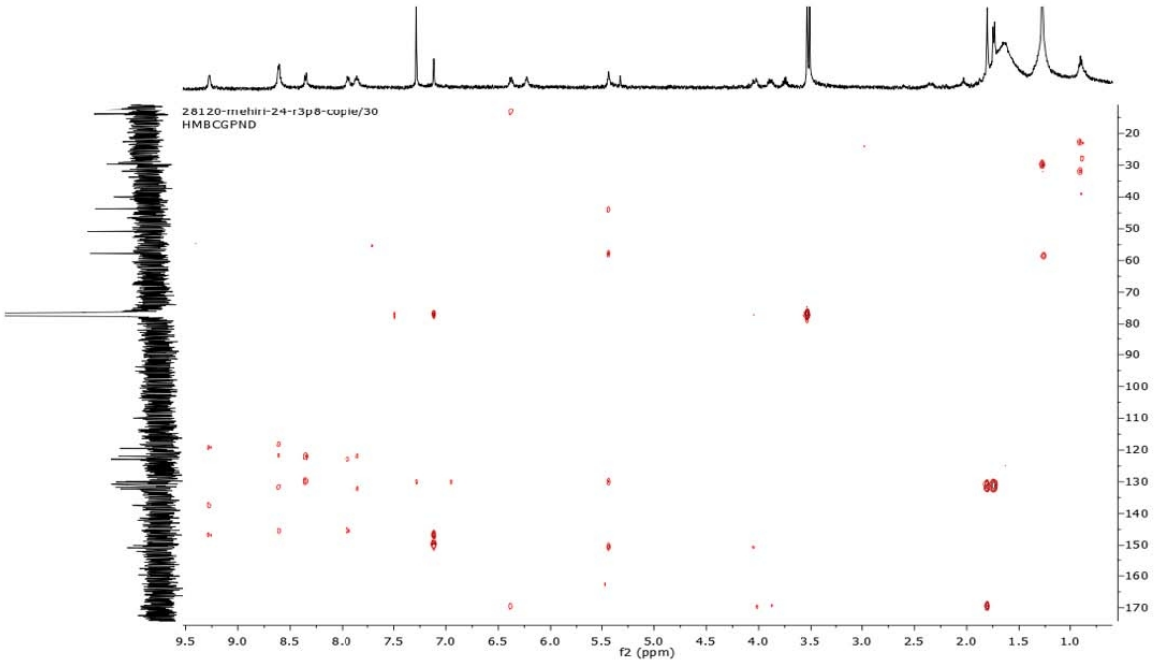

Figure 7. HMBC spectrum of dihydrocystodytin G.

Table 1. NMR data (500/125 MHz, DMSO-d6) for dihydrocystodytine G (4)

N°	δ_C (ppm)/	mult.	δ_H (ppm)/ *J* (Hz)/ mult.			COSY	HMBC
1	130.55	CH	8.13	8.6	d	2	
2	131.49	CH	8.08	7.2	d	1	
3							
4							
4a	121.14	qC					1, 5
4b	133.15	qC					6
5	122.76	CH	9.19	5.2	d	6	6
6	151.94	CH	9.27	5.6	d	5	5
7a	147.88	qC					6, 9
8	183.75	qC					
9	130.29	CH	6.84		s		12
10	151.88	qC					12, 13
10a	151.48	qC					9
10b	119.08	qC					5
11a	145.13	qC					
12	75.01	CH	5.47	4.6	t	13	9
13	43.88	CH_2	3.75		m	12, 14	12
			3.67		m	12, 14	12
14		NH	7.75	6.2	t	13	
15	169.28	qC					14, 18
16	132.30	qC					19
17	128.94	CH	6.12		m	19	18
18	12.92	CH_3	1.57		m		
19	13.88	CH_3	1,54		m	17	17
1′	63.91	CH_3			s		

4. Conclusion

Our efforts in the search for marine bioactive compounds led to an in-depth study of a specimen of the brown tunicate *Cystodytes* sp. collected in Senegalese waters. From this fruitful investigation, a tetracyclic alkaloid, dihydrocystodytine G (4), was isolated alongside three already known compounds: cystodytines B (1), G (2) and L (3). The structural characterization of these metabolites was carried out using a multidisciplinary approach, combining measurement of rotatory power ([α]D), various spectroscopic techniques (1D and 2D NMR, mass spectrometry) and validation against literature data. Given the vital importance of pyridoacridine alkaloids in medicinal chemistry, where they form the basis of many pharmaceutical agents, it is conceivable that the ongoing modulation of the structure of these marine metabolites could reveal promising new candidates for therapeutic applications.

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