

RESEARCH

Open Access

Haliscosamine: a new antifungal sphingosine derivative from the Moroccan marine sponge *Haliclona viscosa*

Belkasssem El-Amraoui^{1*}, Jean-Fançois Biard² and Aziz Fassouane^{1,3}

Abstract

In the aim of searching for new antifungal products from marine origin, we have isolated a sphingosine derivative, (9*Z*)-2-amino-docos-9-ene-1,3,13,14-tetraol (Haliscosamine) from the Moroccan sea sponge *Haliclona viscosa* using bio-guided (antifungal) HPLC methods. The molecular structure of this compound was elucidated by spectrometric techniques IR, UV, MS and NMR. The isolated metabolite showed a significant antifungal activity against *Cryptococcus* and *Candida* species and a weak general toxicity in the brine shrimp lethality test. Further research is needed to study its *in vivo* activity, as well as to elucidate the mechanism underlying its activity in the hope of a future use in medical mycology.

Keywords: Haliscosamine; *Haliclona*; *Candida*; *Cryptococcus*

Introduction

Our laboratory aims to find new antifungal metabolites from marine origin for use in human medicine on one hand, and on the other hand in phytopathology. Marine invertebrates of Moroccan Atlantic coast are our preferred source of producers of active substances, specifically sponges, known generally to contain secondary metabolites with interesting biological activities (Faulkner 2002) including antimicrobial (Baker et al. 2009), antifungal (Clark et al. 2001), antileishmanial (Dube et al. 2007), antioxidant (Regoli et al. 2004) and cytotoxic activities (Ayyad et al. 2009, Fusetani et al. 1989, Erickson et al. 1997, Rashid et al. 2000). In the first work (El-Amraoui et al. 2010), we screened antifungal activity in hydroalcoholic and organic extracts of 14 sponges and showed that three species of them are active against pathogenic fungi and bacteria: *Haplosclerida adocia*, *Cinachyrella tarentina* and *Haliclona viscosa*. This latter species being shown the most active, we chose it to isolate the active compound. Kupchan partitioning, then multistep HPLC from the organic extract provided a pure active product. We determined its structure and evaluated its antifungal potential and its toxicity.

Results

Isolation of the active product

Lyophilized sponge (800 g) was extracted with EtOH, the extract partitioned between CH₂Cl₂ and H₂O, and the organic solution submitted to a Kupchan liquid partition procedure. EtOAc and MeOH:H₂O fractions were pooled, then the mixture was successively separated by three steps of HPLC to yield 47 mg of an amorphous pale yellow product. All steps of this isolation were bio-guided by antifungal (agar disc-diffusion) test. The total mass of the product (taking into account other fractions containing isolated compound) was estimated to be 80 mg from 800 g of dry sponge (0.01%).

Molecular structure of the product isolated from *Haliclona viscosa*

The molecular formula of the compound was determined to be C₂₂H₄₅NO₄, indicating one degree of unsaturation, by HRESIMS with the molecular ion peaks at *m/z* 410.3255 ([M + Na]⁺) for C₂₂H₄₅NO₄Na (calculated 410.32463, δ 2ppm), and *m/z* 388.3431 ([M + H]⁺) for C₂₂H₄₆NO₄ (calculated 388.34268, δ 1ppm) in positive ion mode. MS analysis of the product ([M + H]⁺, 388) showed fragments at 370, 352, 334, 316 for the loss of four -OH, and 299 for the -NH₂. Same analysis was done with the acetylated product showing a [M + Na]⁺ ion at *m/z* 620

* Correspondence: elamraouibelkasssem@yahoo.fr

¹Faculty of Science, University of Chouaib Doukkali, El-Jadida, Morocco
Full list of author information is available at the end of the article

consistent with a penta-acetylated product, and fragments at 560, 500, 440 and 380 for the losses of four acetyl moieties (Figure 1). No ion at 320 for a fifth acetyl was observed.

The UV spectrum (in MeOH) (Figure 2) showed weak absorptions ($\delta=1178$ at 207.5 nm and $\delta=228$ at 281.0 nm).

The IR spectrum (Figure 3) showed absorptions at 3358, 1065 and 1048 (hydroxyl), 2926 and 2855 (aliphatic), 1659 and 1630 cm^{-1} (double bond). No peak was visible for a carbonyl function.

Analysis of ^1H (Figure 4), ^{13}C (Figure 5) and HSQC (in deuterated methanol CD_3OD) (Figure 6) experiments revealed the presence of 22 carbons with 39 protons, including one methyl, 15 methylenes and six methines. Table 1 shows NMR spectroscopic data of compound. The presence of four oxygenated C atoms at 59.11 (C-1), 67.72 (C-3), 73.36 (C-13), 73.81 (C-14), a probably nitrogenated C atom at 57.72 (C-2), and two double

bonded carbons at 129.08 (C-9) and 129.82 (C-10) were elucidated. General appearance of the NMR spectra suggested that it is a derivative of the sphingosine. Therefore, the six remaining protons should correspond to one $-\text{NH}_2$ and four $-\text{OH}$. From the careful examination of the 2D NMR experiments spectra (HMQC, HMBC (Figures 7, 8, 9 and 10), COSY (Figures 11 and 12), TOCSY (Figure 13)), three different structural units were identified: C-1 to C-5, C-8 to C-16 and C-20 to C-22 (Figure 14). However, respective positions of the carbons 9/10, 8/11 and 13/14 were not clear because there was a strong overlapping of their signals on the ^1H -NMR spectrum. Experiments with other solvents were limited by the poor solubility of compound in AcN, CH_2Cl_2 and CHCl_3 . Finally, the assignments of the ^1H -NMR signals were determined by recording a new set of experiments with increased resolution for the corresponding areas, and by extensive ^1H -NMR decoupling experiments (Figures 15, 16, 17 and 18). Consequently,

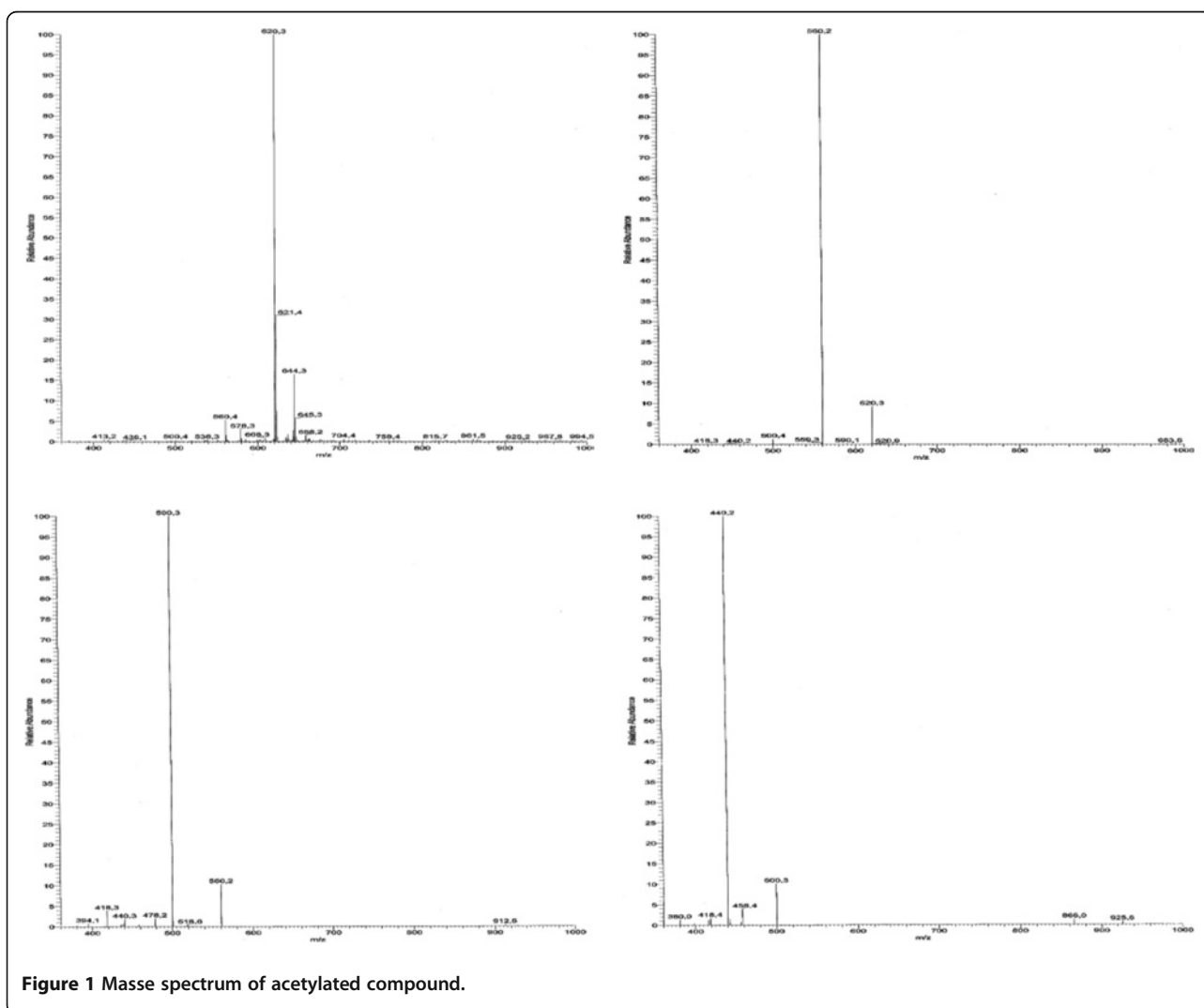


Figure 1 Masse spectrum of acetylated compound.

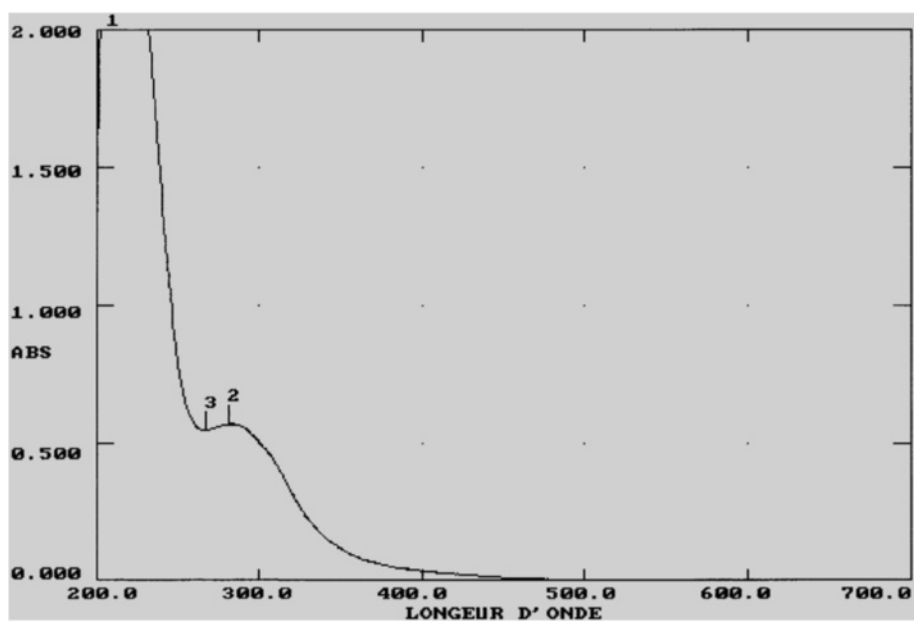


Figure 2 UV-spectrum of haliscosamine.

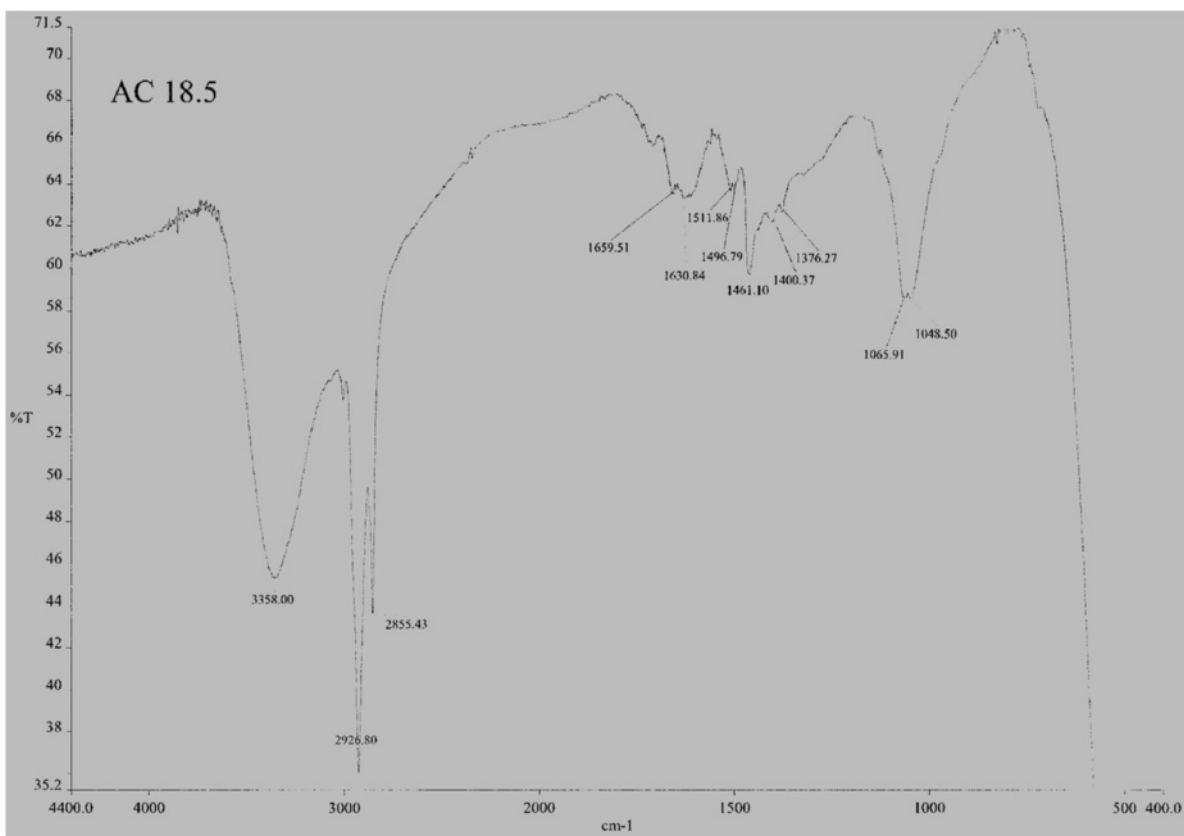


Figure 3 IR spectrum of haliscosamine.

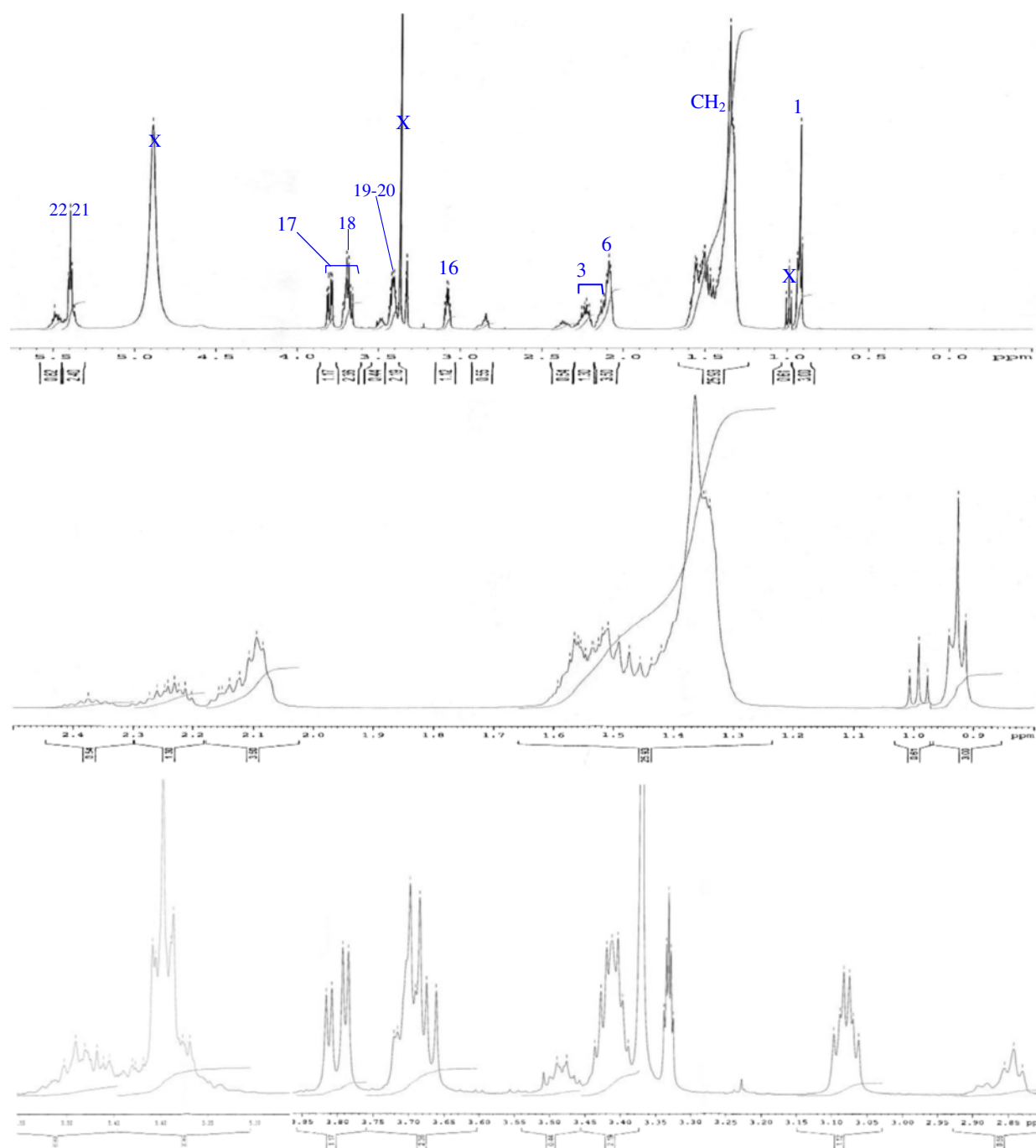


Figure 4 ^1H NMR spectrum of haliscosamine.

clear relationships between all carbons were established for these three partial structures.

Assemblage of the three sub-structures was done by supplementary ^1H -NMR decoupling experiments, which allow to link C-22 to C-21 and C-20, then to C-16 by the way of the three CH_2 at 29.17, 29.18 and

29.20 (10, 9, 8 respectively). Accordingly, the two last remaining $-\text{CH}_2$ (C-6 and C-7) should be positioned between C-5 and C-8 to complete the structural elucidation of compound. Geometry of the double bond was assigned to be Z on the basis of the ^1H -NMR experiments: Irradiations at δ 2.09 (Figure 19) (H' s of C-8) and 2.28

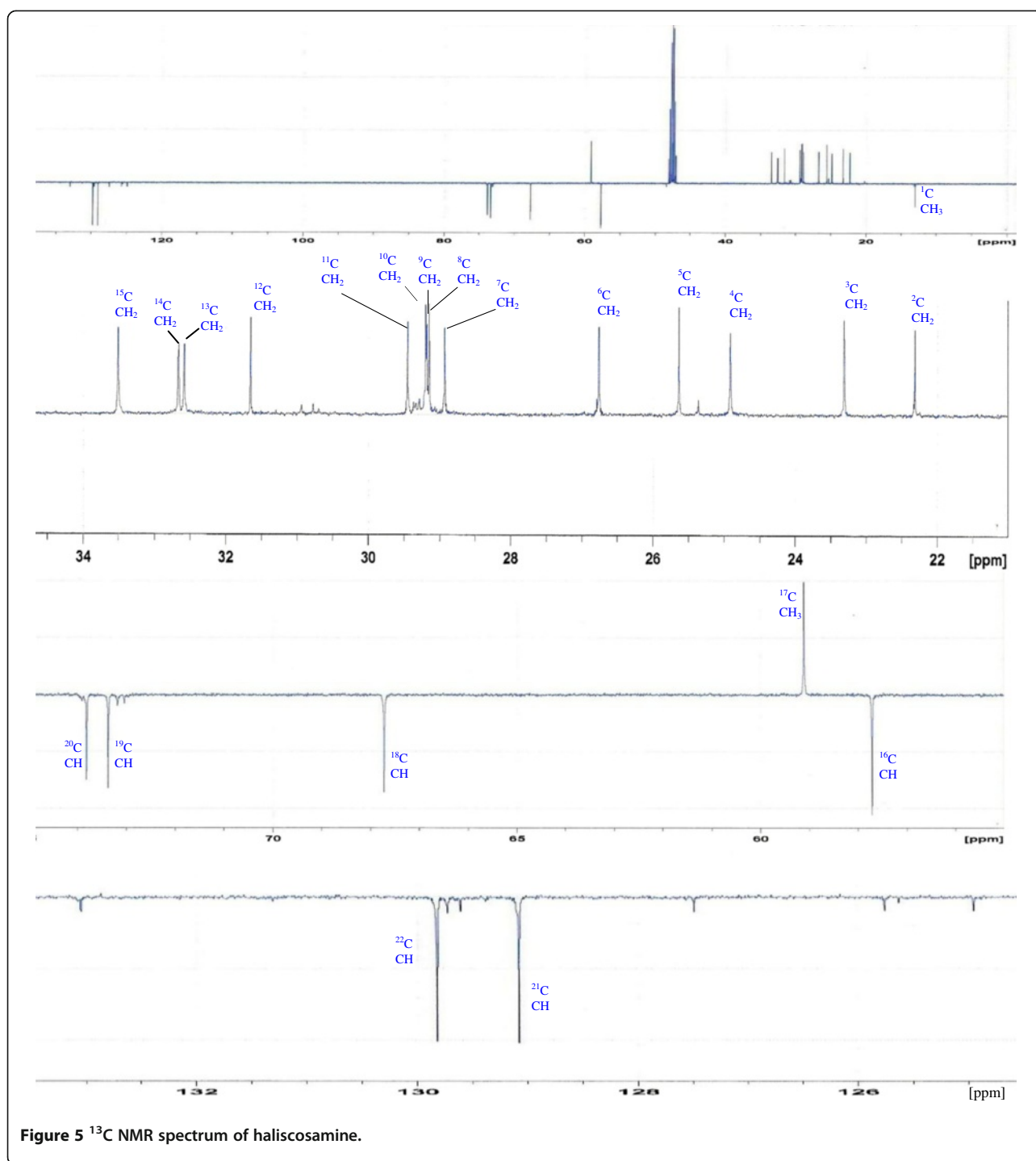


Figure 5 ^{13}C NMR spectrum of halicosamine.

(Figure 20) ($\text{H}'\text{s}$ of C-11) resulted in a change of the olefinic protons group and allowed to found a 10 Hz coupling constant for these protons. Recording of other ^1H -NMR spectrum in CD_3OD with some drops of deuterated benzene (C_6D_6) (Figure 21) increases the resolution and allowed confirming this 10 Hz coupling constant. The geometry of the double bond was confirmed

by the weak difference between the chemical shifts values of the carbons C-8 (26.77) and C-11 (23.32).

The optical rotation was $[\alpha]_{\text{D}}^{20} = 22,3$ ($c = 0,76$, MeOH). The compound isolated from *H. viscosa*: (9Z)-2-amino-docos-9-ene-1,3,13,14-tetraol (Figure 22), is a new sphingosine derivative. Therefore, we named this product halicosamine.

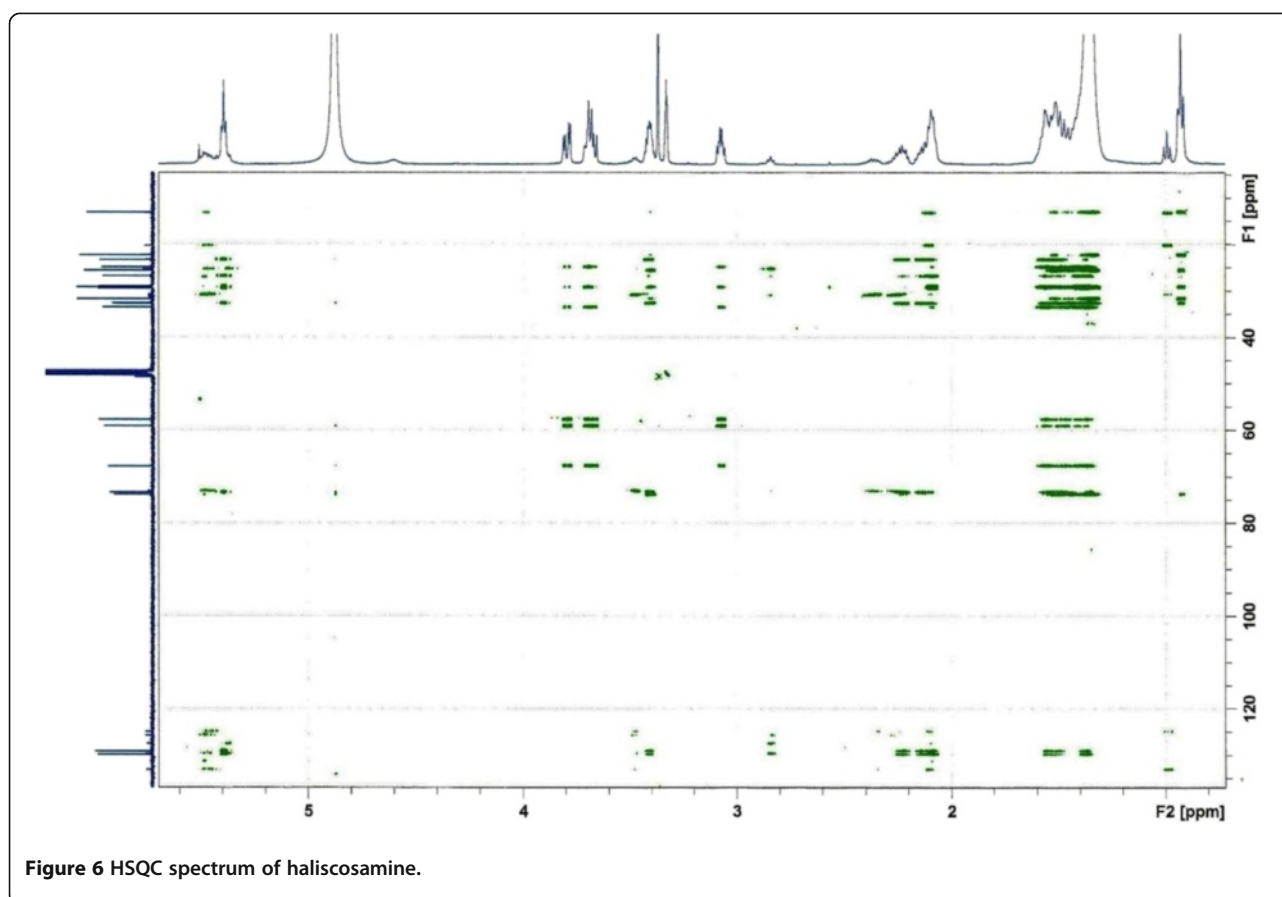


Figure 6 HSQC spectrum of haliscosamine.

Biological activities of haliscosamine

Antifungal activity

In vitro antifungal activity and minimal inhibitory concentration (MIC₉₀) of haliscosamine against the pathogenic yeasts were reported in Tables 2 and 3 respectively.

The *in vitro* antifungal activity by the diffusion method showed that haliscosamine was more active than nystatin in inhibiting the growth of *Cryptococcus neoformans* (ATCC 11576), but showed the same activity as nystatin in inhibiting the growth of *C. albicans* (ATCC 10231). On the other hand, haliscosamine was active against *C. tropicalis* (R2 CIP 1275.81), an amphotericin B and nystatin resistant strain.

The MIC₉₀ of haliscosamine is less than that of nystatin on *C. neoformans* and *C. albicans*, and the same level as the previous in *C. tropicalis*.

Toxicity test

Lethality concentration (24 h-LC₅₀ value) for haliscosamine is 664.86 µg/mL. This general toxicity activity is considered weak when the LC₅₀ was between 500 and 1000 µg/mL, moderate when the LC₅₀ was between 100 and 500 µg/mL, and designated as strong when the LC₅₀ ranged from 0 to

100 µg/mL (Padmaja et al. 2002, Canales et al. 2007). Consequently, the toxicity of the haliscosamine may be considered as weak by this test.

Discussion

Haliscosamine isolated from the Moroccan marine sponge *Haliclona viscosa* is a new derivative of sphingosine with an original molecular structure ((*Z*)-2-amino-docos-9-ene-1,3,13,14-tetraol, C₂₂H₄₅NO₄). However, the relative and absolute stereochemistries of the molecule remain to be determined, with confirmation by total synthesis.

The high content (0.01%) of the haliscosamine in the sponge, as its presence in the free state and not involved in a ceramide or other complex lipid is remarkable. The haliscosamine is added to products already isolated from sponges of the genus *Haliclona*, such the alkaloids viscosamine (Volk and Kock 2003), viscosaline (Volk and Kock 2004) and haliclamine A, B, C and D (Fusetani et al. 1989, Volk et al. 2004). The activity of this product also shows the bioactive potential of marine sponges from the Atlantic Coast of Morocco, and encourages them to continue our sorting activity. The only study that has been done before has been the isolation of untenospongine B (antimicrobial) from the marine sponge *Hippospongia*

Table 1 NMR spectroscopic data for haliscosamine

Carbon	$\delta^{13}\text{C}$ ppm	$\delta^1\text{H}$ ppm $J(\text{Hz})$	HMBC	COSY	TOCSY
1a	59.12, CH ₂	3.67dd ($J = 11.7, 4.0$)	2, 3	1b, 2, 3	2, 3
1b		3.79dd ($J = 11.7, 4.0$)	2, 3	1a, 2, 3	2, 3
2	57.72, CH	3.07 m ($J = 6.8, 4.0$)	1, 3, 4	1	1, 3
3	67.72, CH	3.69 m ($J = 6.8, 4.0$)	1, 2, 4, 5	1, 4	1, 2, 4, 6
4a	33.51, CH ₂	1.47 m	2, 3, 5		
4b		1.57 m	2, 3, 5		
5a	24.92, CH ₂	1.40 m	3		
5b		1.57 m	3		
6	28.94, CH ₂	1.37 m			
7	29.45, CH ₂	1.37 m	8	8	9
8	26.77, CH ₂	2.09 m	7, 9	7, 9, 10	
9	129.08, CH	5.39 m	8	8, 10	8, 7
10	129.82, CH	5.40 m	11	8, 9, 11	11, 13
11a	23.32, CH ₂	2.13 m	10, 12, 13	10, 12	10, 13
11b		2.24 m	10, 12, 13	10, 12	10, 13
12a	32.59, CH ₂	1.54 m	11, 13		13
12b		1.59 m	11, 13		13
13	73.37, CH	3.42	11, 12, 14, 15		10, 11, 12, 15
14	73.81, CH	3.40	13, 15, 16	15	
15a	32.67, CH ₂	1.44 m	13, 14, 16	14	13
15b		1.54 m	13, 14, 16	14	13
16a	25.64, CH ₂	1.37 m	14, 15		
16b		1.52 m	14, 15		
17	29.17, CH ₂	1.37 m			
18	29.18, CH ₂	1.37 m			
19	29.20, CH ₂	1.37 m			
20	31.66, CH ₂	1.33 m	21, 22		
21	22.32, CH ₂	1.36 t ($J = 6.5$)	20, 22		
22	13.06, CH ₃	0.92	20, 21		

communis (Rifai et al. 2004) and of fasciculatin (cytotoxic and inhibitor of lymphocyte proliferation) isolated from *Ircinia variabilis* (Rifai et al. 2005).

Haliscosamine has a remarkable antifungal effect. Sphingolipids are already known for their antiseptic and antifungal activity (Bibel et al. 1995), and sphingosine and its derivatives are natural antimicrobial agents, protecting the human skin from bacterial colonization (Bibel et al. 1993) as well as being anti-inflammatory agents (Radhika et al. 2005).

Compared to nystatin, haliscosamine showed *in vitro* significant activity against *Candida albicans* (ATCC 10231), *Candida tropicalis* (CIP 1275.81) and *Cryptococcus neoformans* (ATCC 11576). These three yeasts are often involved in human mycology especially *C. tropicalis* that is resistant to nystatin and amphotericin B.

Comparing the antifungal activity of haliscosamine with (-)-untenospongine B isolated from the marine sponge *Hippospongia communis* collected from the Atlantic Coast of Morocco (Rifai et al. 2004), it was found that haliscosamine was more active than untenospongine B in inhibiting the growth of *C. tropicalis* (R2 CIP 1275.81), *C. albicans* (ATCC 10231) and *C. neoformans* (ATCC 11576) (Table 2). So, haliscosamine is an interesting product, thanks to its antifungal potential against strains involved in human mycology, including those resistant to nystatin. Further studies are still needed to determine *in vivo* activity and toxicity of haliscosamine in the animal model as well as to elucidate the mechanism underlying its activity. The lethal concentration (24 h-LC₅₀ = 664.86 $\mu\text{g/mL}$) of haliscosamine is greater than 500 $\mu\text{g/mL}$, so the general toxicity activity was regarded weak. Therefore, if the

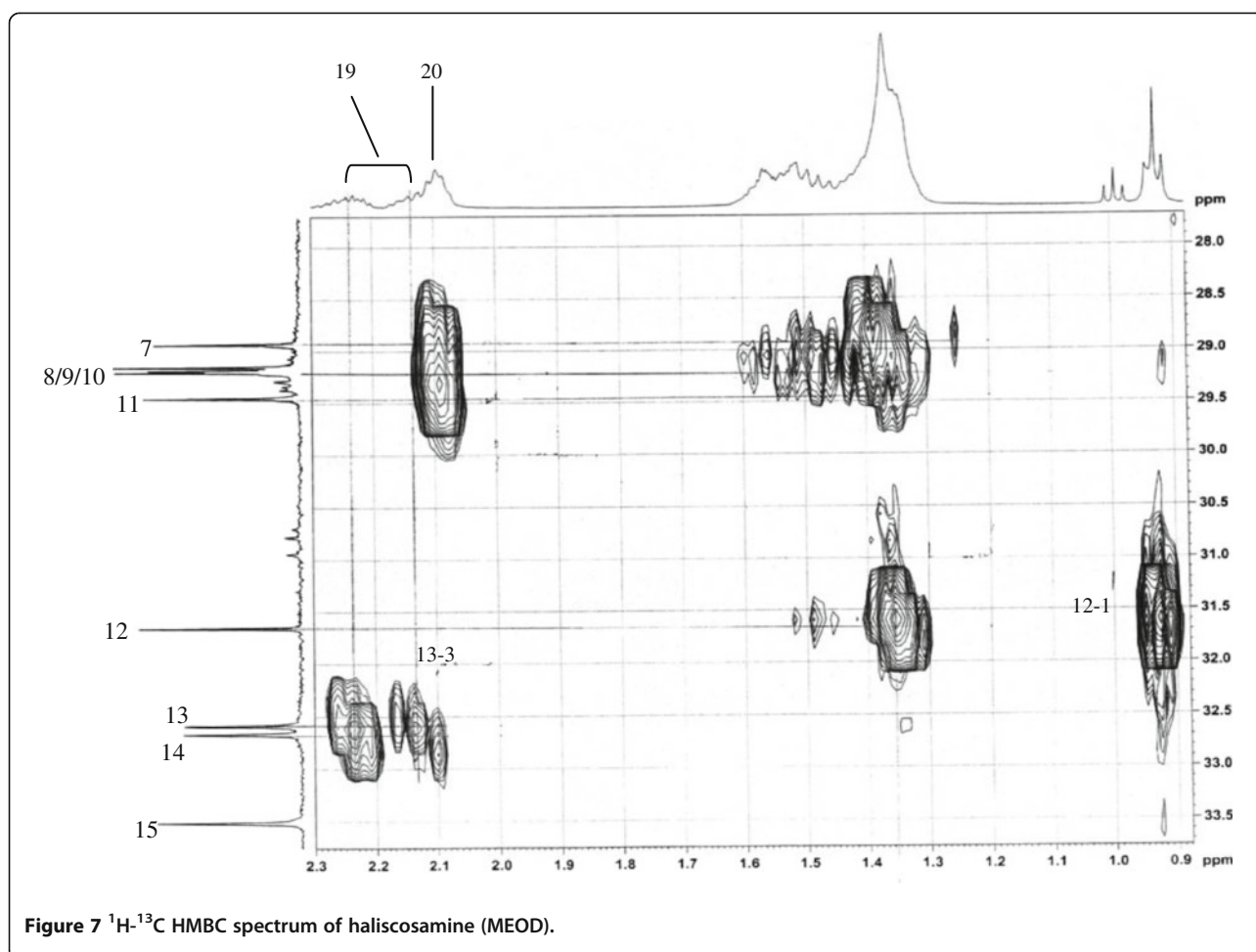


Figure 7 ^1H - ^{13}C HMBC spectrum of haliscosamine (MEOD).

activity persists *in vivo*, then the product could be a candidate to assess more fully for a development of a new drug for the treatment of fungal infections.

Materials and methods

Biological marine material

The marine sponge (Ref. EM14) *H. viscosa* (Topsent, 1888) was collected from the Atlantic coast of El-Jadida city, Morocco (N 33 15 422, W 008° 29 722) (El-Amraoui et al. 2010). The sponge was identified by Dr. Maria-Jesús Uriz, Research Professor at the Centro de Estudios Avanzados de Blanes(CEAB), Spain. After collection, the sponge was immediately cut into small pieces, washed with sterile distilled water, then frozen at -30°C for two days and immediately freeze-dried to give a lyophilized material ready for extraction.

General experimental procedures

Freeze-dryer was a FreeZone2.5Plus type (Labconco, USA).

TLC was carried out on precoated Macherey-Nagel Alugram silica, and spots were visualized by spraying with iodine vapor or ninhydrine reagents.

The UV spectrum was recorded on a Helios Omega spectrophotometer (Thermo Scientific, France).

The IR spectrum was obtained on a spectrometer IR-FT Paragon 1000 PC (Perkin-Elmer, USA).

The NMR spectra (^1H , ^{13}C , HSQC, HMBC, COSY and TOCSY experiments) were recorded on a Bruker 500 with a TXI crysonde (PRISM, Rennes University, France) and a Bruker Avance 500 (CRMPO, Rennes University, France) for in-deep 2D experiments and irradiations tests.

HRESIMS data were recorded on a Micromass Zab Spec Tof (CRMPO, Rennes University, France). ESIMS data of the acetylated product was recorded on a Finnigan LCQ mass spectrometer (IFREMER, Nantes, France).

Optical rotation was measured on a Schmidt + Haensch Polartronic NH8 polarimeter.

Acetylation: 1 mg of isolated product was treated with 1 mL of acetic anhydride (Ac_2O):pyridine (1:1 v/v) for

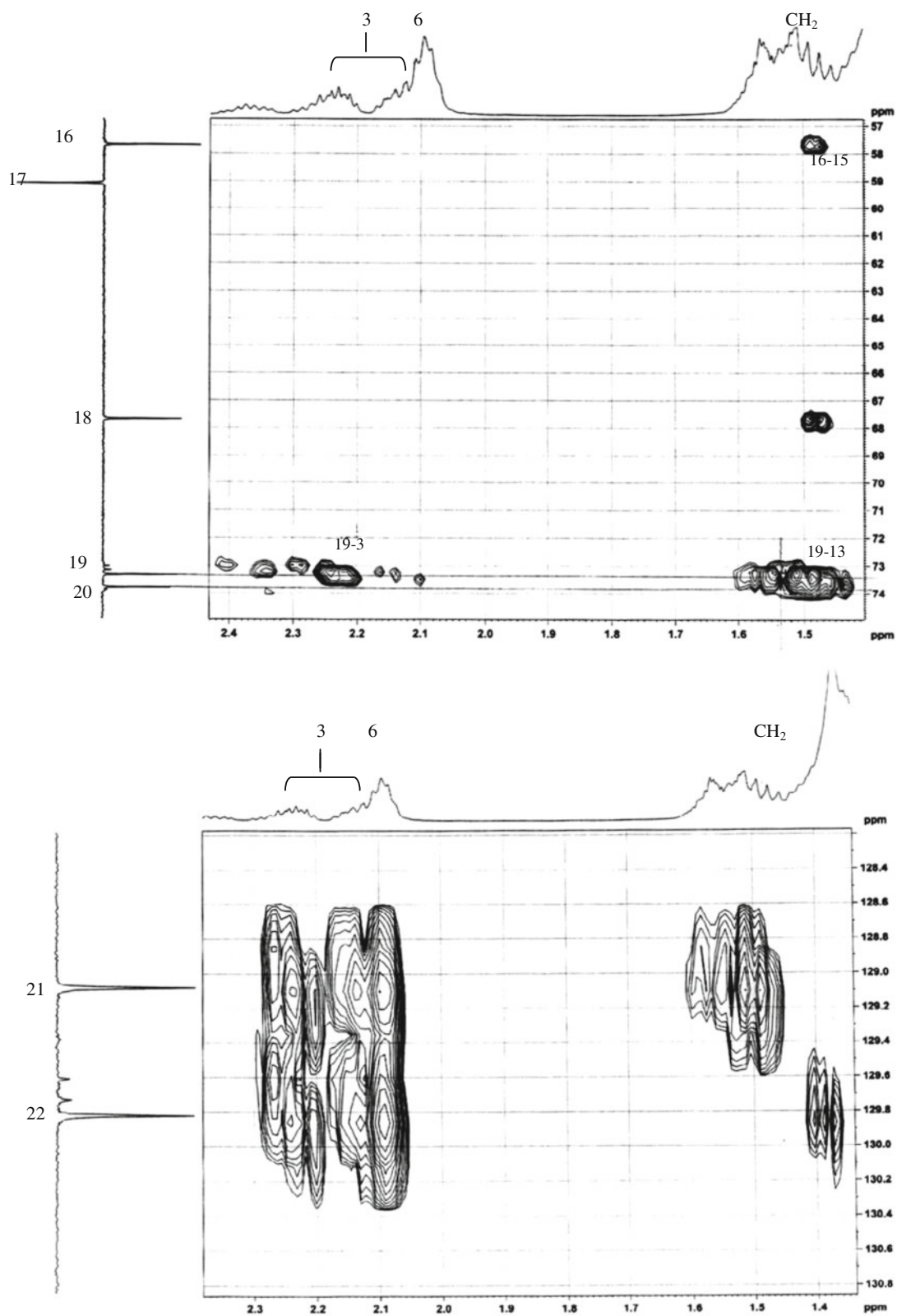


Figure 8 ^1H - ^{13}C HMBC spectrum of haliscosamine (MEOD).

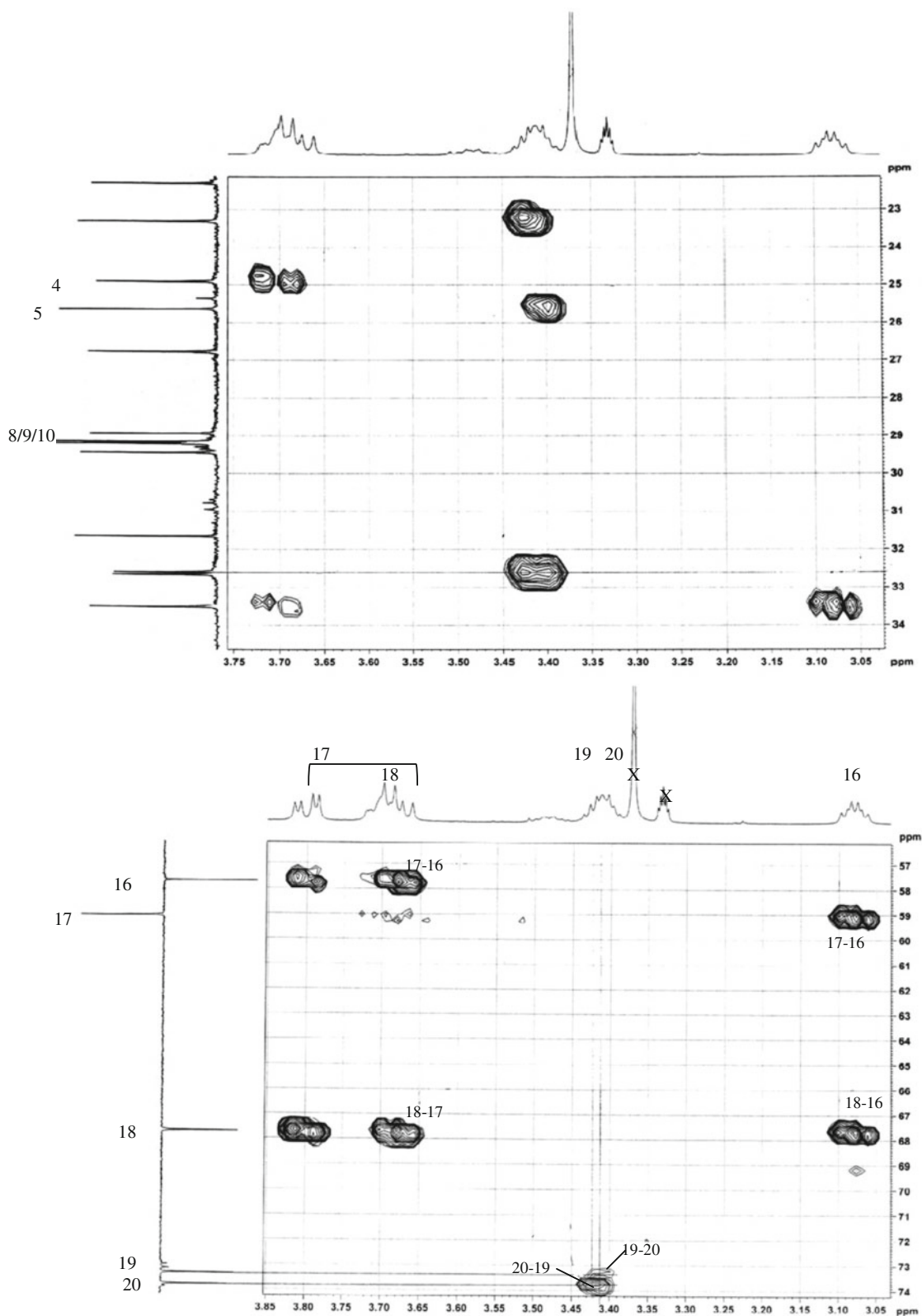


Figure 9 ^1H - ^{13}C HMBC spectrum of haliscosamine (MEOD).

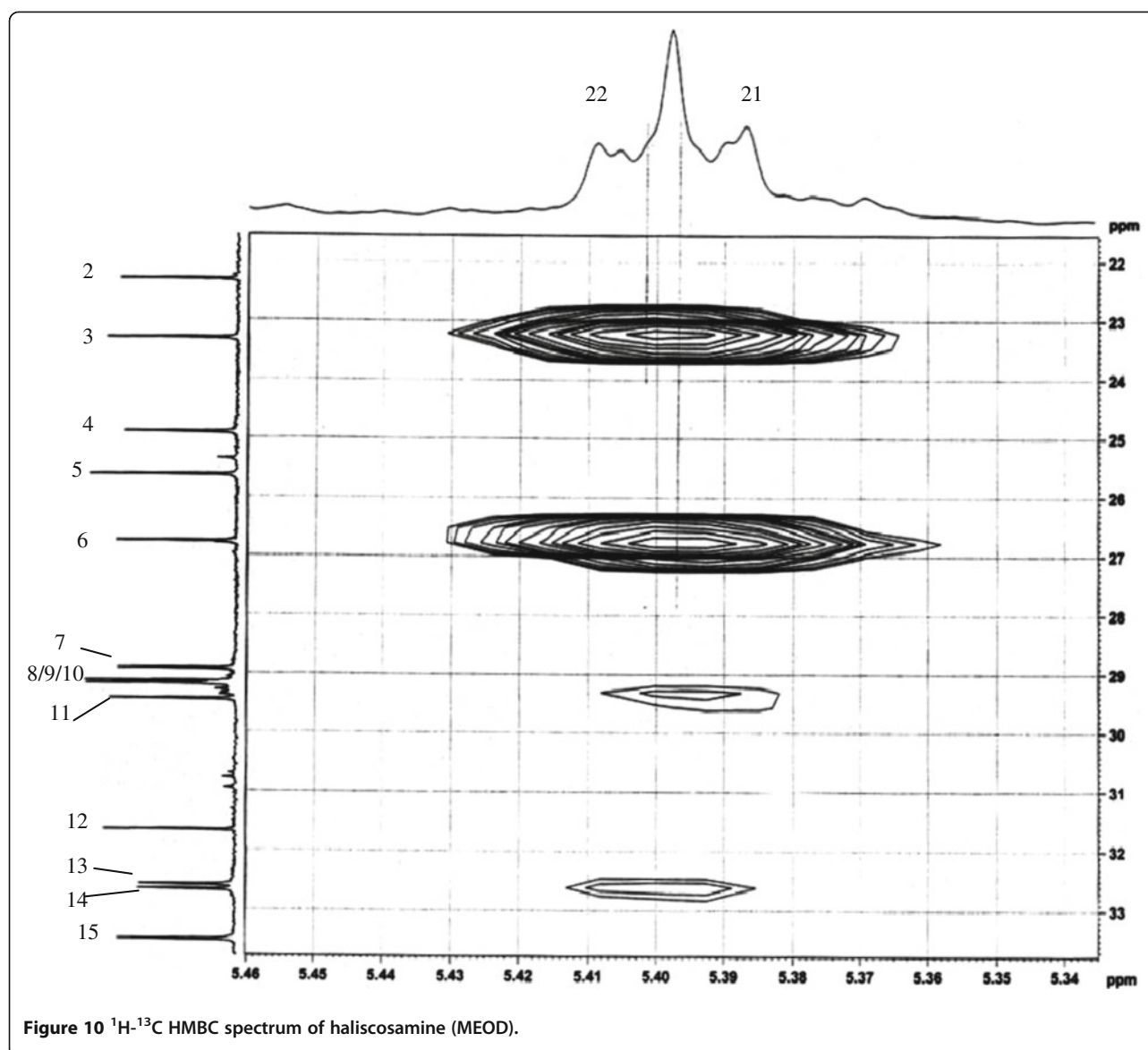


Figure 10 ^1H - ^{13}C HMBC spectrum of haliscosamine (MEOD).

24 h at ambient temperature, then 40 mL of +4°C water was added and the mixture extracted with diethyl-ether (4×10 mL) to afford 1.2 mg of acetylated product.

Extraction and isolation

The sample (800 g of lyophilized sponge) was homogenized with ethanol 80% (1×1000 mL), allowed to stand in a dark chamber for 24 h and filtered. The residue was again extracted with absolute ethanol (2×1000 mL). Both ethanolic extracts were combined, and then evaporated at reduced pressure until total evaporation of ethanol. The resulting aqueous suspension was completed with distilled water to 1000 mL as final volume and extracted with CH_2Cl_2 (3×500 mL). The CH_2Cl_2 solutions were

combined, dried on anhydrous sodium sulphate (Na_2SO_4), filtered and concentrated at reduced pressure to give a dichloromethane extract (3 g).

This extract was fractionated by modified Kupchan method: 3 g were dissolved in 300 mL of $\text{MeOH}:\text{H}_2\text{O}$ (9:1 v/v) and extracted with 3×150 mL of hexane (A) (1.5 g). The remaining solution was adjusted with distilled water to get the proportions 6:4 v/v, then extracted with 3×150 mL of dichloromethane (B) (0.3 g) and then with 3×150 mL of ethyl acetate (C) (0.7 g). The residue was lyophilized (D) (0.5 g).

Fractions C and D were combined (1.2 g), applied on silica gel 60 (25 g) column, and eluted with successive mixtures (110 mL) of CHCl_3 : MeOH (10:0; 9:1; 8:2; 7:3; 6:4; 4:6 and 0:10 v/v) to yield nine fractions. Fractions 4, 5

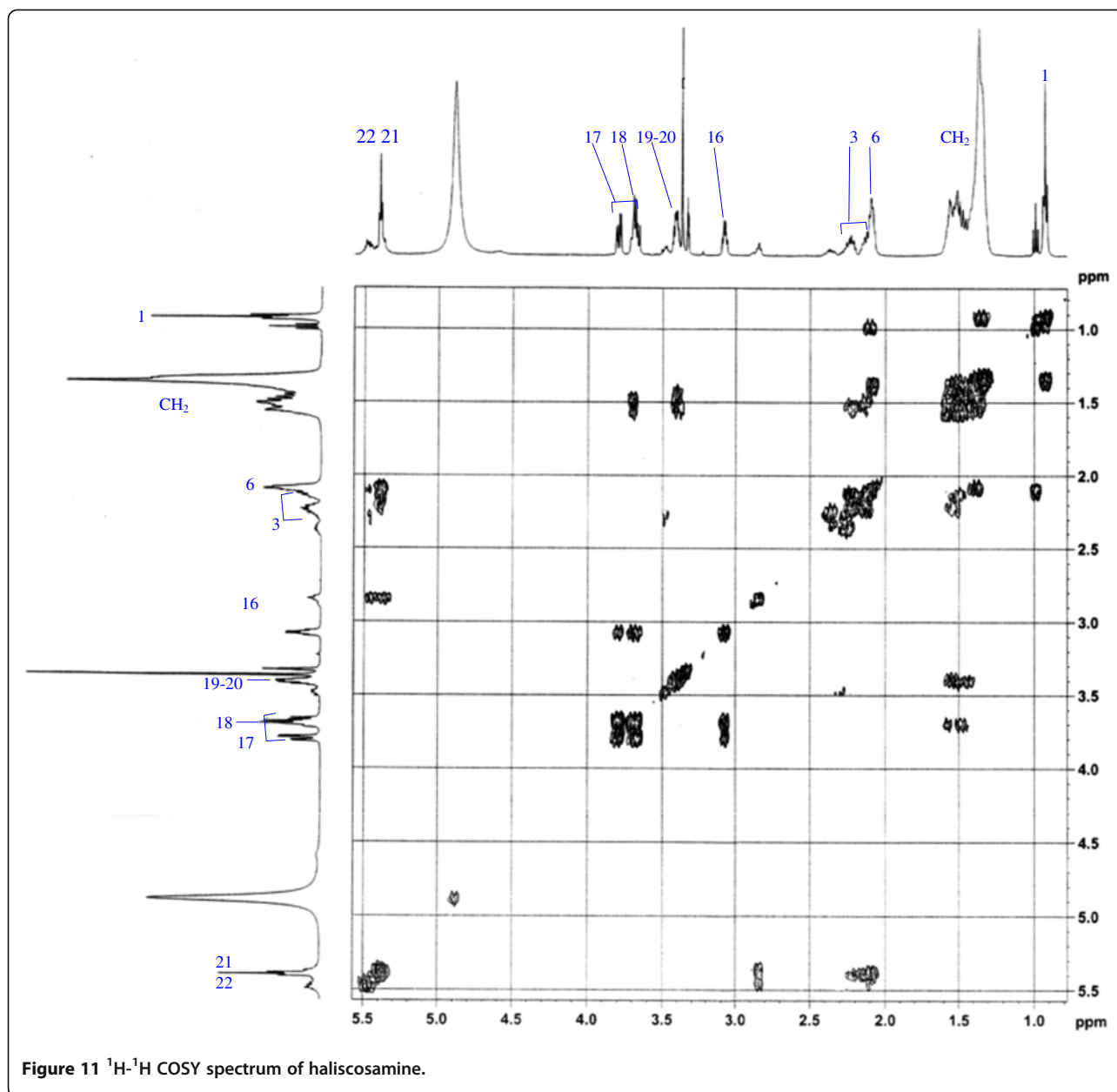


Figure 11 ^1H - ^1H COSY spectrum of halicosamine.

and 6 were pooled (260 mg), then separated on a semi-preparative HPLC diol column (Inertsil Diol L10OH.25R, 10×250 mm, $10 \mu\text{m}$) by a CH_2Cl_2 : MeOH (8.5:1.5 v/v) isocratic mixture. Purification of subfractions 2 (95 mg) by HPLC C18 column (Inertsil ODS-3, 4.6×250 mm, $5 \mu\text{m}$) with isocratic MeOH:H₂O (6.5:3.5 v/v) gave 47 mg of a pure active product.

Microorganisms

The fungal species obtained from the Fungi Culture Collection (FCC) of the National Cultures Collection of Microorganisms of the Pasteur Institute, Paris, France,

from the Collection of Institut Pasteur (CIP) and from the American Type Culture Collection (ATCC) were used as the antifungal test strains: *Candida albicans* (ATCC 10231), *Candida tropicalis* (R2 CIP 1275.81, an amphotericin B and nystatin resistant strain), *Cryptococcus neoformans* (ATCC 11576). The yeasts were maintained on the Sabouraud's agar medium at 28°C.

Antifungal activity

The antifungal activity was assessed *in vitro* by agar disc-diffusion test. The minimum inhibitory concentration was evaluated by the microdilution method.

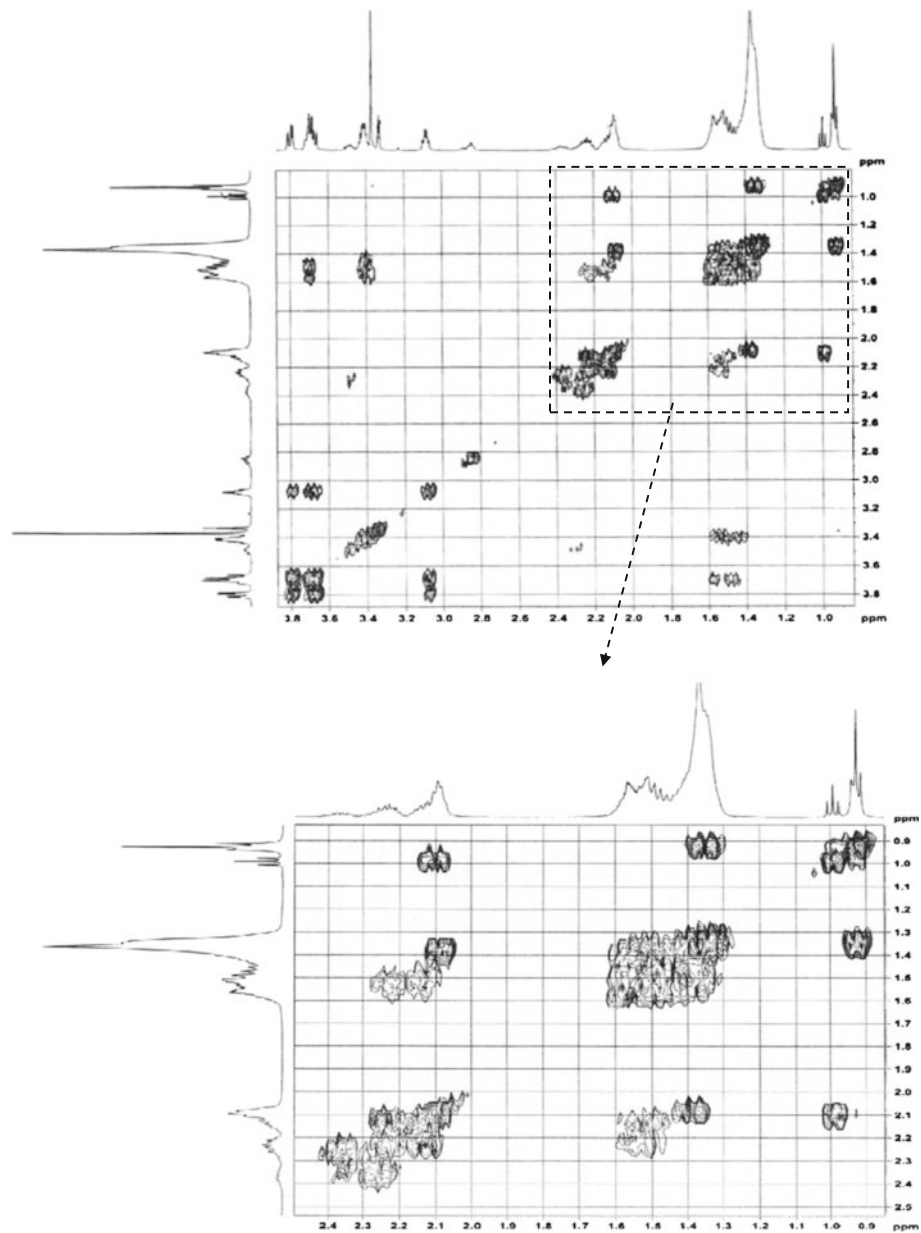


Figure 12 Detail of ^1H - ^1H COSY spectrum of haliscosamine.

Agar disc-diffusion test

This test uses Yeast Morphological Agar (YMA) as medium [yeast nitrogen base (Difco) 60.5 g/L; asparagin (Prolabo) 1.5 g/L; glucose (Merck) 10 g/L and agar (Merck) 20 g/L]. The suspensions of yeast were adjusted in sterile water to match the density of a 0.5 McFarland Standard. Each disk received 100 μg of sponge extract and was applied on the test media which were previously inoculated with each test strain. Plates were first kept at 4°C for at least two hours to allow the diffusion of chemicals, and then incubated at 28°C. Inhibition zones were measured after 24 h of

incubation (Galeano and Martínez 2007). Standard disks of nystatin (100 μg) served as positive antifungal control. All the assays were carried out in triplicate.

Minimum inhibitory concentration (MIC)

The MIC₉₀ (the lowest concentration causing at least 90% of growth inhibition when compared to drug-free control) of the isolated compound was measured using the method described by Rifai et al. (2005). Yeast Morphological Broth medium was used as test media. Tests

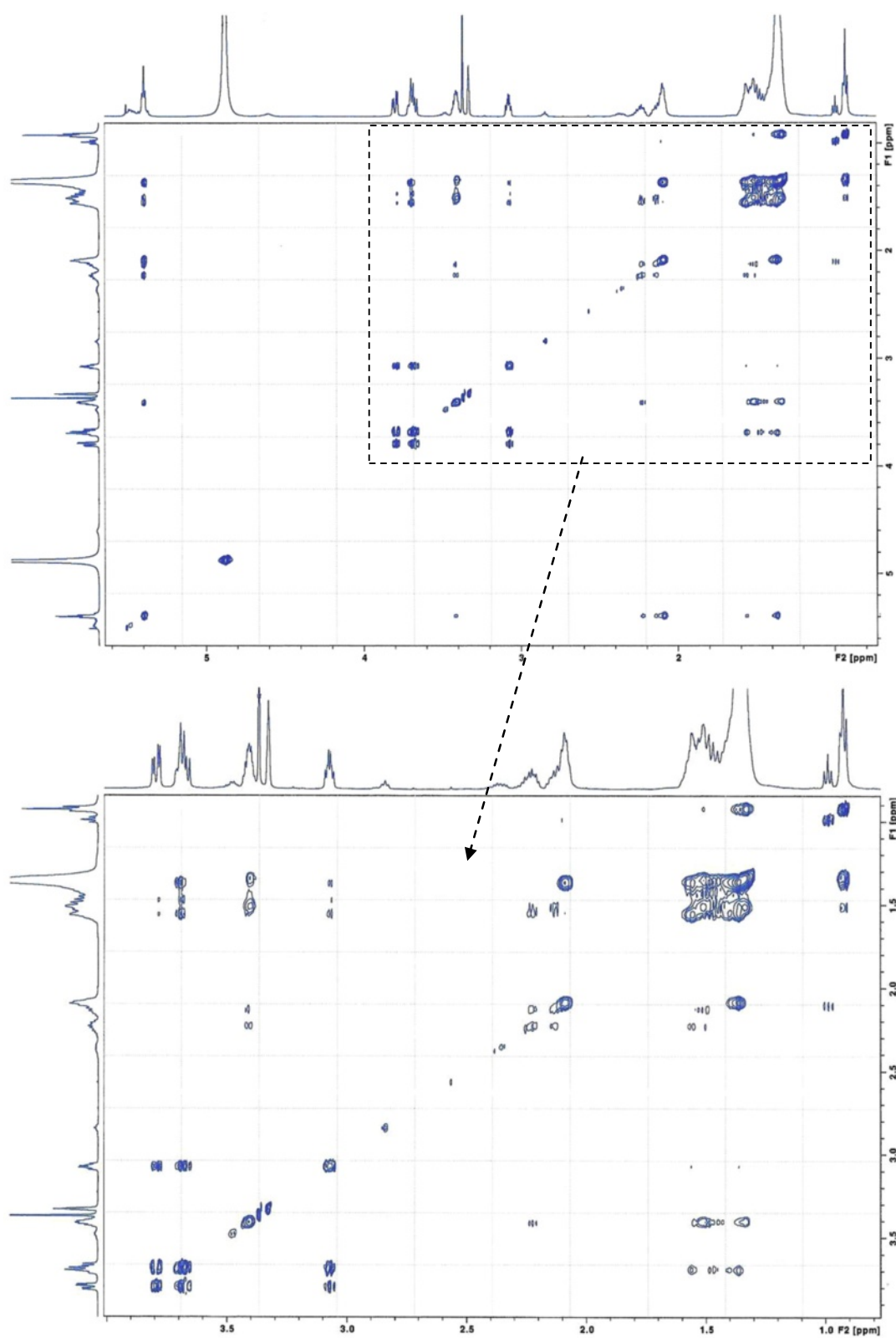


Figure 13 TOCSY spectrum of haliscosamine.

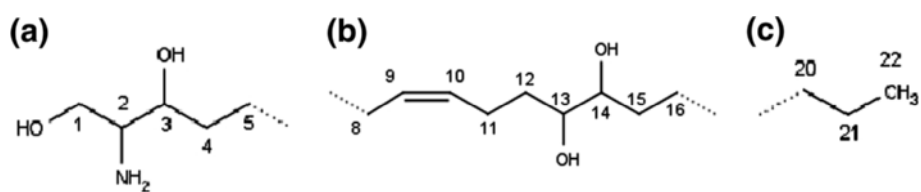


Figure 14 The three sub-structures of haliscosamine. (a) C-1 to C-6, (b) C-8 and C-16 (c) C-20 to C-22.

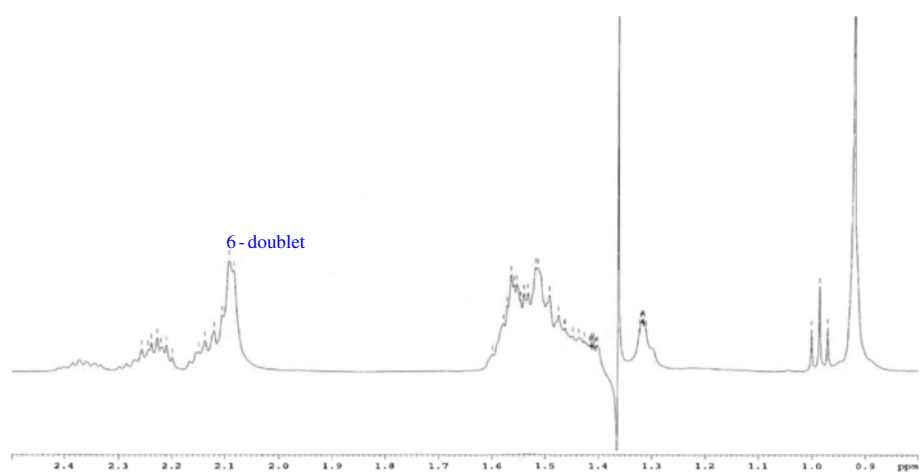


Figure 15 ^1H spectrum of haliscosamine (MEOD, irradiation at δ 1.369 ppm).

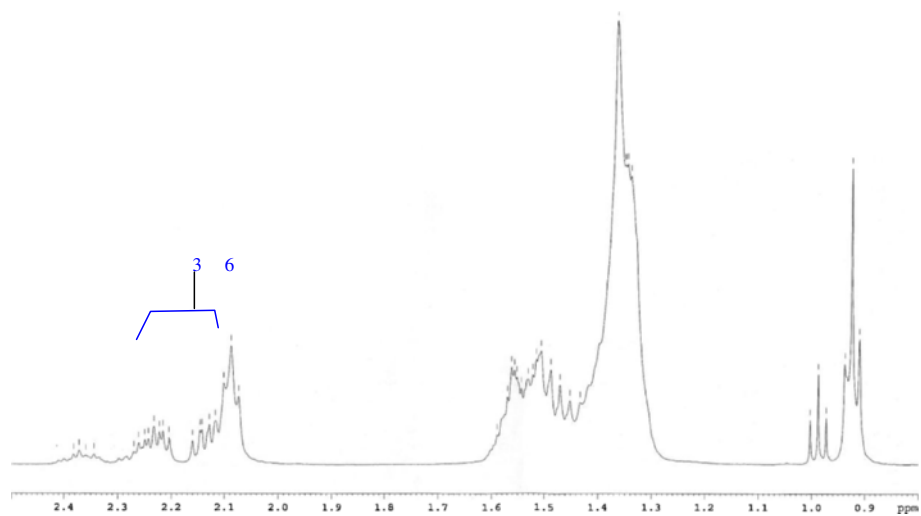


Figure 16 ^1H spectrum of haliscosamine (MEOD, irradiation at δ 5.398 ppm).

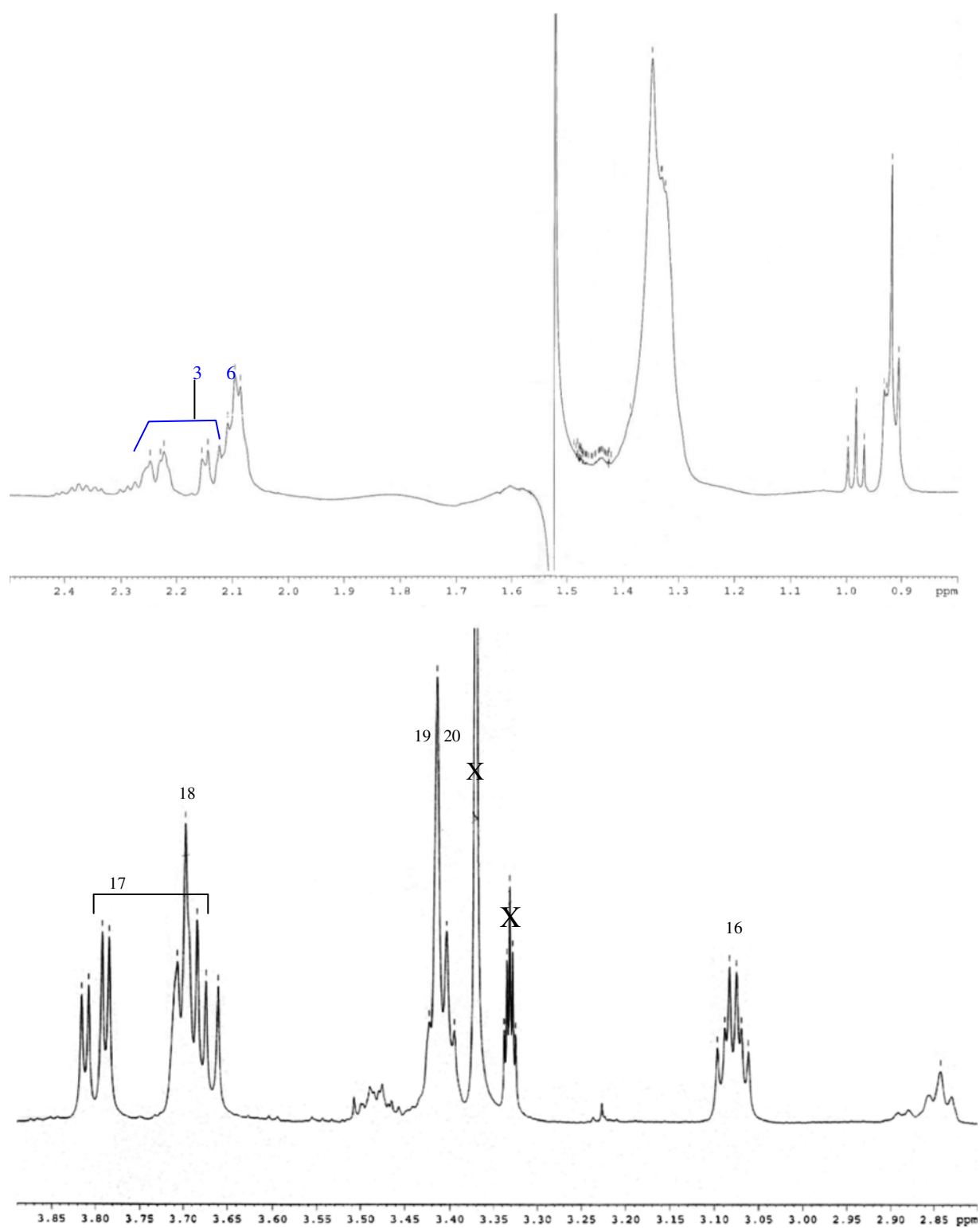


Figure 17 ¹H spectrum of haliscosamine (MEOD, irradiation at δ 1.523 ppm).

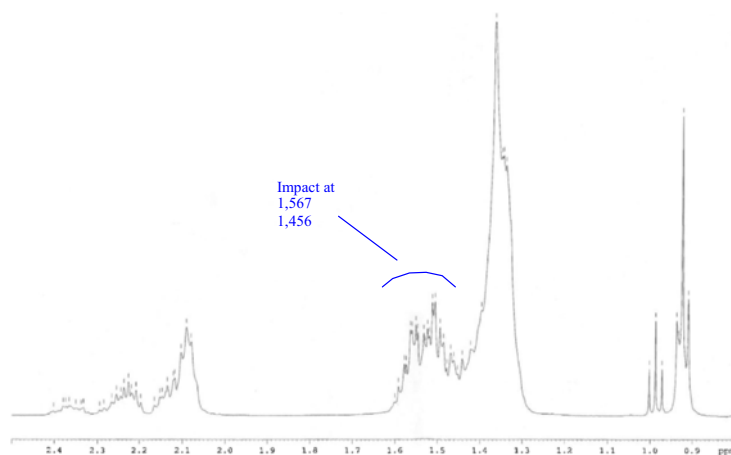


Figure 18 ^1H spectrum of haliscosamine (MEOD, irradiation at δ 3.415 ppm).

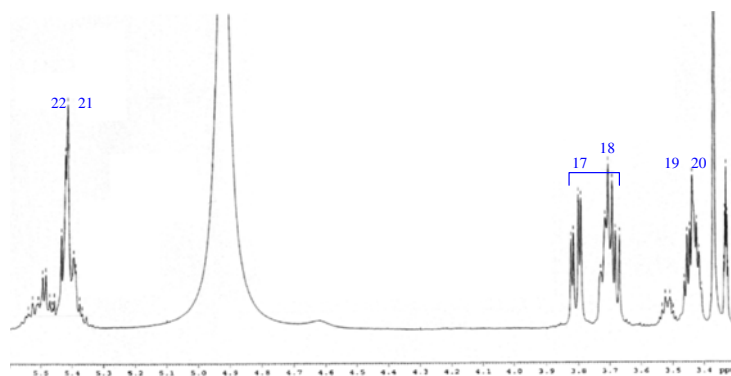


Figure 19 ^1H spectrum of haliscosamine (MEOD, irradiation at δ 2.09).

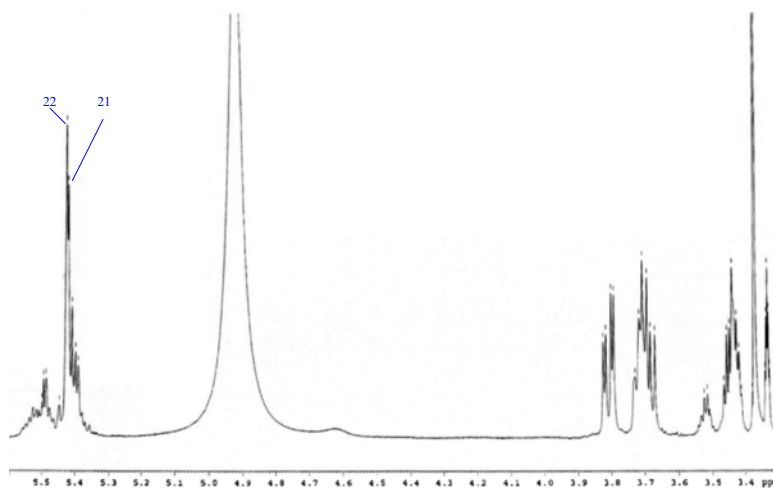


Figure 20 ^1H spectrum of haliscosamine (MEOD, irradiation at δ 2.28).

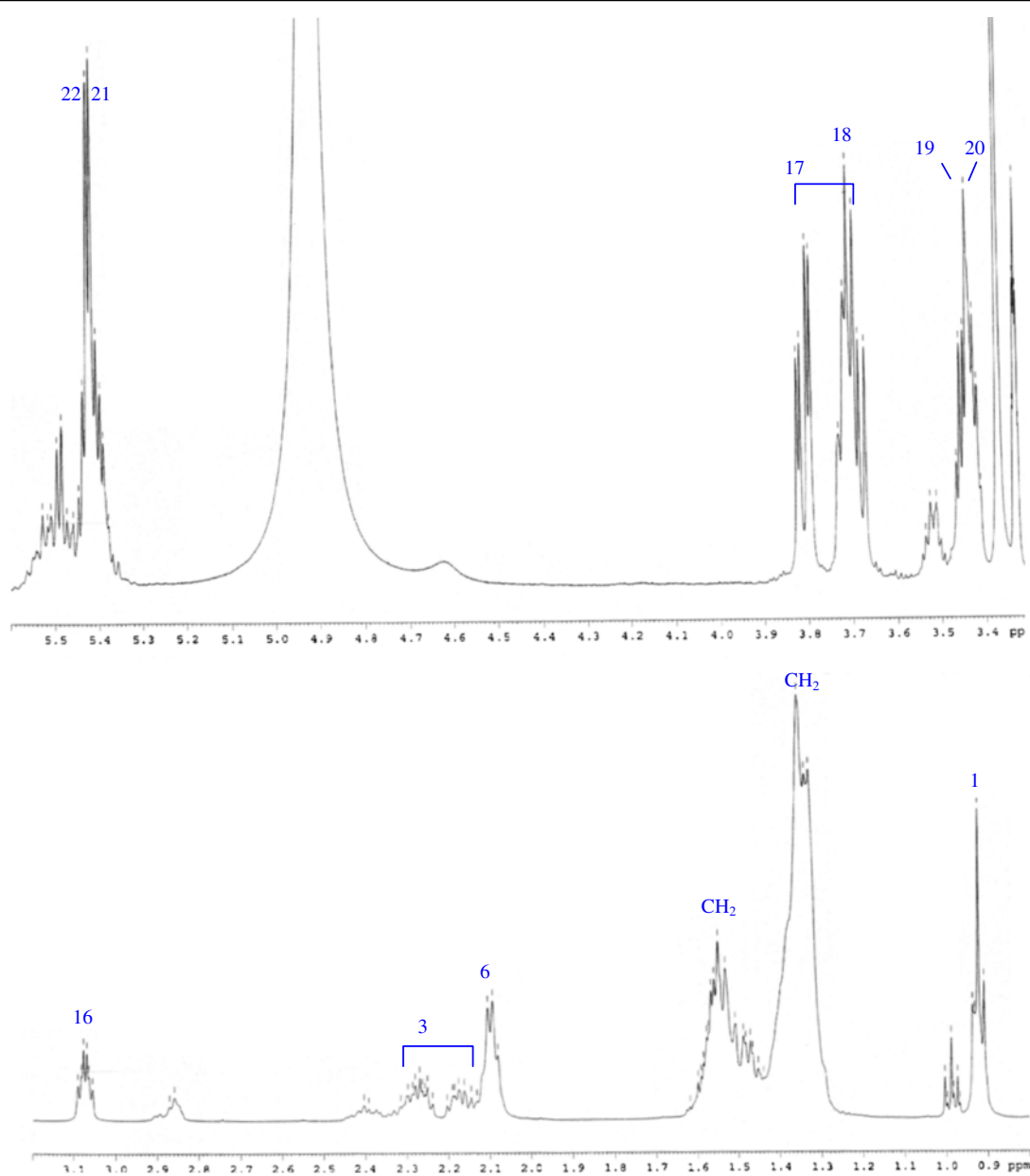


Figure 21 ¹H NMR spectrum of halicosamine (CD₃OD + few drops of C₆D₆).

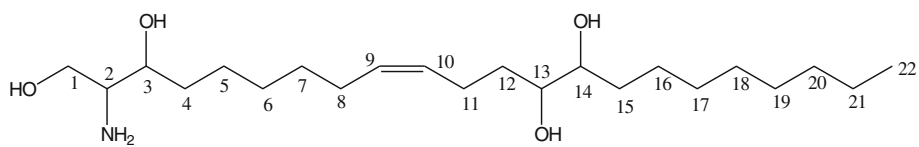


Figure 22 Molecular structure of halicosamine [(9Z)-2-amino-docos-9-ene-1,3,13,14-tetraol].

Table 2 *In vitro* antifungal activity of haliscosamine and nystatin against pathogenic yeasts

Test organism	Growth inhibition diameter (mm)		
	Haliscosamine (100 µg)	Nystatin (100 µg)	(-)-untenspongini B (100 µg)
<i>C. neoformans</i>	28	24	0
<i>C. albicans</i>	25	25	17
<i>C. tropicalis</i>	25	0	5

were performed in 96-well round bottom sterile culture plates. The suspensions of yeast were adjusted in sterile water to match the density of a 0.5 McFarland Standard. The wells of a microdilution plate were inoculated with 180 µL of the culture medium containing a final inoculum of 0.5 - 2.5 10³ CFU/mL. The test drug and positive control (nystatin) previously solubilized in dimethylsulphoxide (DMSO) were serially diluted two fold in the liquid medium to give a range of concentration from 640 to 0.1 µg/mL. Twenty µL of each concentration were added to wells containing culture suspension except the growth control well. The final concentration ranged from 64 to 0.01 µg/mL. Plates were incubated at 35°C for 48 h. Fungal growth was assessed at 494 nm by measuring the optical density in each well using an enzyme immunoassay multiwell reader (Sigma diagnostic). The test was carried out in triplicate.

Brine shrimp toxicity test

Hatching shrimp

Brine shrimp eggs (*Artemia salina*) are hatched in artificial seawater prepared by dissolving sea salt in distilled water (38 g/L) during 48 h incubation in a warm room (22 - 29°C). Seawater is placed in a small unequally divided tank and shrimp eggs are added to the larger compartment of the tank which is covered by aluminium foil to darken it. The illuminated compartment attracts shrimp larvae (*nauplii*) through perforations in the dam (Meyer et al. 1982).

Brine shrimp microwell toxicity assay

The toxicity of haliscosamine was monitored by the brine shrimp lethality test. Samples were dissolved in DMSO and diluted with sea water so that the final

Table 3 Minimum Inhibitory Concentration (MIC₉₀) of haliscosamine and nystatin against pathogenic yeasts

Test organism	Minimum inhibitory concentration (µg/mL)	
	Haliscosamine	Nystatin
<i>C. neoformans</i>	0.2 - 0.4	3.12 - 6.25
<i>C. albicans</i>	0.4 - 0.8	3.12 - 6.25
<i>C. tropicalis</i>	0.4 - 0.8	0

concentration of DMSO did not exceed 0.05%. Serial dilutions (2000, 200 and 20 µg/mL) of samples were made in wells of 96-well microplates in triplicate in 100 µL of sea water (Rahman et al. 2001). The last row was left with sea water and DMSO only served as the drug free control. 100µL of suspension of *nauplii* containing about 10 larvae were added into each well and incubated for 24 h at 22-29°C. The plates were then examined under a binocular microscope (× 12.5) and the number of dead *nauplii* in each well was counted. One hundred µL of methanol were then added and after 10 min, the total numbers of shrimp in each well was counted and recorded. Lethality concentration fifties (LC₅₀ values) for each assay were calculated by taking the average of the three experiments using a Finney Probit analysis program on an IBM computer.

Competing interest

All authors declare that they have no competing interest.

Authors' contributions

All authors read and approved the final manuscript.

Acknowledgements

We thank Dr. Maria-Jesús Uriz, Research Professor at the Centro de Estudios Avanzados de Blanes (CEAB), Spain for sponge identification, Pr. Sourisak Sinbandhit and Pr. Philippe Jehan from CRMPO and Pr. Arnaud Bondon from PRISM platform at Rennes University, France for the NMR and MS, and the CNRST of Morocco for the support of the mobility of the researchers of this project.

Author details

¹Faculty of Science, University of Chouaib Doukkali, El-Jadida, Morocco. ²MMS Research Group, Faculty of Pharmacy, University of Nantes, Nantes, France. ³Director of National School of Business and Management (ENCG), El Jadida, Morocco.

Received: 14 January 2013 Accepted: 28 March 2013

Published: 4 June 2013

References

- Ayyad SEN, Bahaffi SOS, Hashish NE (2009) Isolation and structure determination of the biologically active sphingolipids from marine sponge *Haliclona* species. *Nat Prod Res* 23(1):44-50. doi:10.1080/14786410701768246
- Baker PW, Kennedy J, Dobson AD, Marchesi JR (2009) Phylogenetic diversity and antimicrobial activities of fungi associated with *Haliclona simulans* isolated from Irish coastal waters. *Mar Biotechnol* (NY) 11(4):540-547. doi:10.1007/s10126-008-9169-7
- Bibel DJ, Aly R, Shah S, Shinefield HR (1993) Sphingosines: antimicrobial barriers of the skin. *Acta Derm Venereol* 73(6):407-411
- Bibel DJ, Aly R, Shinefield HR (1995) Topical sphingolipids in antiseptic and antifungal therapy. *Clin Exp Dermatol* 20(5):395-400
- Canales M, Hernandez T, Serrano R, Hernandez LB, Duran A, Rios V, Sigrist S, Hernandez HL, Garcia AM, Angeles-Lopez O, Fernandez-Araiza MA, Avila G (2007) Antimicrobial and general toxicity activities of *Gymnosperma glutinosum*: a comparative study. *J Ethnopharmacol* 110(2):343-347. doi:10.1016/j.jep.2006.10.002
- Clark RJ, Garson MJ, Hooper JN (2001) Antifungal alkyl amino alcohols from the tropical marine sponge *Haliclona n. sp.* *J Nat Prod* 64(12):1568-1571
- Dube A, Singh N, Saxena A, Lakshmi V (2007) Antileishmanial potential of a marine sponge, *Haliclona exigua* (Kirkpatrick) against experimental visceral leishmaniasis. *Parasitol Res* 101(2):317-324. doi:10.1007/s00436-007-0469-z
- El-Amraoui B, Biard JF, Uriz MJ, Rifai S, Fassouane A (2010) Antifungal and antibacterial activity of porifera extracts from the Moroccan atlantic coasts. *J Mycol Med* 20(1):70-74. doi:10.1016/j.mycmed.2009.11.001

- Erickson KL, Beutler JA, Cardellina IJ, Boyd MR (1997) Salicylilalamides A and B, novel cytotoxic macrolides from the marine sponge *Haliclona* sp. *J Org Chem* 62(23):8188–8192
- Faulkner DJ (2002) Marine natural products. *Nat Prod Rep* 19(1):1–48
- Fusetani N, Yasumuro K, Matsunaga S, Hirota H (1989) Haliclamines A and B, cytotoxic macrocyclic alkaloids from a sponge of the genus *Haliclona*. *Tetrahedron Lett* 30(49):6891–6894. doi:10.1016/S0040-4039(01)93381-7
- Galeano E, Martínez A (2007) Antimicrobial activity of marine sponges from urabá gulf, Colombian Caribbean region. *J Mycol Med* 17(1):21–24. doi:10.1016/j.mycmed.2006.12.002
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982) Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med* 45(1):31–34
- Padmaja R, Arun PC, Prashanth D, Deepak M, Amit A, Anjana M (2002) Brine shrimp lethality bioassay of selected Indian medicinal plants. *Fitoterapia* 73(6):508–510
- Radhika P, Rao PR, Archana J, Rao NK (2005) Anti-inflammatory activity of a new sphingosine derivative and cembrenoid diterpene (lobohedleolide) isolated from marine soft corals of *Sinularia crassa* TIXIER-DURIVault and *Lobophytum* species of the Andaman and Nicobar Islands. *Biol Pharm Bull* 28(7):1311–1313
- Rahman A-U, Choudhary MI, Thomsen WJ (2001) Bioassay techniques for drug development. Harwood academic, British library, UK
- Rashid MA, Gustafson KR, Boswell JL, Boyd MR (2000) Haligramides A and B, two new cytotoxic hexapeptides from the marine sponge *Haliclona nigra*. *J Nat Prod* 63(7):956–959
- Regoli F, Nigro M, Chierici E, Cerrano C, Schiapparelli S, Totti C, Bavestrello G (2004) Variations of antioxidant efficiency and presence of endosymbiotic diatoms in the Antarctic porifera *Haliclona dancoi*. *Mar Environ Res* 58(2–5):637–640. doi:10.1016/j.marenvres.2004.03.055
- Rifai S, Fassouane A, Kijjoo A, Soest RV (2004) Antimicrobial activity of untenospongin B, a metabolite from the marine sponge *Hippospongia communis* collected from the Atlantic coast of morocco. *Mar Drugs* 2:147–153
- Rifai S, Fassouane A, Pinho PM, Kijjoo A, Nazareth N, São M, Nascimento J, Herz W (2005) Cytotoxicity and Inhibition of lymphocyte proliferation of Fasciculatin, a linear furanosesterterpene Isolated from *Ircinia variabilis* collected from the Atlantic Coast of Morocco. *Mar Drugs* 3:15–21
- Topsent E (1888) Contribution à l'étude des Clionides. *Archives de Zoologie expérimentale et générale* 5 bis(2):1–165, pls I-VII
- Volk Christian A, Lippert H, Lichte E, Köck M (2004) Two New haliclamines from the arctic sponge *Haliclona viscosa*. *Eur J Org Chem* 2004(14):3154–3158. doi:10.1002/ejoc.200400026
- Volk CA, Kock M (2003) Viscosamine: the first naturally occurring trimeric 3-alkyl pyridinium alkaloid. *Org Lett* 5(20):3567–3569. doi:10.1021/ol035006i
- Volk CA, Kock M (2004) Viscosoline: new 3-alkyl pyridinium alkaloid from the Arctic sponge *Haliclona viscosa*. *Org Biomol Chem* 2(13):1827–1830. doi:10.1039/b403413a

doi:10.1186/2193-1801-2-252

Cite this article as: El-Amraoui et al.: Haliscosamine: a new antifungal sphingosine derivative from the Moroccan marine sponge *Haliclona viscosa*. *SpringerPlus* 2013 **2**:252.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Immediate publication on acceptance
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com