

Chapter 2

Bioactive Molecules of Marine Invertebrates from South-West Indian Ocean: Status and Perspectives

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Abstract Marine invertebrates produce a large number of unique and structurally diversified natural products which represent a major source of bioactive molecules, particularly for pharmaceuticals leads. This great potential has elicited worldwide scientific and economic interests in searching novel drugs from marine invertebrates. These efforts have resulted in several thousands of novel marine natural products exhibiting a wide range of bioactivities such as anticancer, antiviral, antifungal, and antibacterial properties. To date, the pioneering countries in marine natural products discovery such as USA, Japan, Australia, and Spain are benefiting from the great commercial and social value of such research. Since the early 1990s,

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the Chemistry Laboratory of Natural Substances and Food Sciences (LCSNSA, University of La Réunion) has been trying to gain a foothold in this field of research. The laboratory which is located at Reunion Island is at a strategic location for the research of natural bioactive molecules. Indeed, with a series of islands scattered in the western Indian Ocean along the southeast coast of Africa (Madagascar, Seychelles, the Comoros, Mayotte, Mauritius, Eparses islands), Reunion Island belongs to a global biodiversity hotspot. This paper will therefore provide an outline of the contribution made by the LCSNSA to marine natural products research in the west of Indian Ocean. Over the last 15 years, our research programs were more precisely concentrated on marine invertebrates from Reunion Island, Mayotte, and Madagascar. Among the numerous marine invertebrates encountered in these areas, sponges, ascidians, and soft corals have predominated in all our collection expeditions and have therefore received special attention from our research group. More than 100 new compounds showing relevant bioactivity were isolated. Among these compounds, a series of guanidine alkaloids, designated netamines A-S were isolated from a Madagascan sponge, *Biemna laboutei*. Absolute configurations of netamines I and J determined via a joint experimental and theoretical circular dichroism study are also discussed in this paper.

Keywords Marine invertebrates • Bioactive molecules • Absolute configuration • Circular dichroism • Indian Ocean

2.1 Introduction

Traditionally, the search of new drug candidates from nature is conducted on terrestrial plants and microorganisms. However, since the 1960s, because of the increasing needs for drugs to control new illnesses or resistant strains of microorganisms, the concept of drugs from the sea attracted some interest. The marine environment, particularly in tropical areas, offers indeed a rich diversity of species, which is in many ways comparable to that of tropical rain forests. This environment also contains a wide number of organisms for which there are no terrestrial counterparts. Besides, ecological pressures on marine organisms, which include abiotic factors such as light, temperature, pressure, salinity, currents, chemical composition of sea water, and biotic factors such as competition for space, deterrence of predation and pathogens, ability to successfully reproduce, may have led to the evolution of unique chemical components responsible for these actions and interactions.

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Such compounds, having no role in the basic life process, i.e., being not essential for the growth and the survival of the organism, are called secondary metabolites and their great diversity of structures in marine organisms is one of the most fascinating aspects of marine natural products chemistry. To date, after more than 60 years of intensive research, chemistry of marine natural products has become a mature field. The search for new biomedical products from marine micro- and macro-organisms resulted in the isolation of more than 24,000 new secondary metabolites, many of which possess pharmacodynamic properties [1]. A broad spectrum of biological activities has been detected, such as antibiotic, antifungal, toxic, cytotoxic, neurotoxic, etc. In more recent years, new targets have been added to the general screening, for example AIDS, immunosuppression, anti-inflammation, Alzheimer's disease, aging process, and some tropical diseases.

In addition to their pharmaceutical applications, secondary metabolites may also be employed as chemotaxonomic markers useful for elucidating classification problems and phylogenetic relationships [2]. Taxonomy and classification of marine organisms such as sponges are inherently difficult as they possess rather few morphological characters. Expertise and long experience are therefore usually needed for a good approximation to the problem. Hence, finding new sets of characters, e.g., those of chemical nature, to aid in classification and phylogeny has quickly become obvious. Secondary metabolites with their great diversity have been suggested as a possible alternative to morphological characters, first because they increase in number from year to year, and then because the structural complexity of the molecules promises a large source of new characters. This complementary approach called "chemotaxonomy" or "chemosystematics" is defined as a classification method in relation to the chemical composition of the organisms or more precisely as a classification according to shared compounds or compound groups. Such compounds also called "chemotaxonomic markers" are ideally not only present in all members of the considered taxon but also absent in others. The importance of the latter is frequently underestimated!

Drug discovery and chemotaxonomy are thus two main tracks in marine natural products chemistry which have attracted the attention of numerous biologists and chemists throughout the world. However, despite this intense global interest, the natural products chemistry of marine flora and fauna of the western Indian Ocean is still limited. Fifteen years ago, the Chemistry Laboratory of Natural Substances and Food Sciences (LCSNSA) decided therefore to develop a research program devoted to marine natural products from this neglected region.

2.2 Why Investigate Indian Ocean Water?

Why investigate South-west Indian water? The answer is quite simple. This area is a perfect location for the collection of marine invertebrates. Including Madagascar, the fourth largest island on earth, the independent nations: Seychelles (including Aldabra), the Comoros, Mauritius (including Rodrigues), and the French overseas

departments: Reunion, Mayotte (one of the Comoros) and the Iles Eparses, this part of Indian Ocean is indeed recognized as a biodiversity hotspot. In addition to the great biodiversity, all the islands are served by reliable and frequent air transportation, so that samples can be transported back to the LCSNSA in good conditions.

Several expeditions were led by the LCSNSA, and were concentrated on Madagascar (a total of 14 expeditions), Mayotte (3 expeditions), and obviously Reunion Island. Expeditions around the island were carried out between 1994 and 2006.

Each time, a large-scale random collection of samples of sub-tidal benthic marine organisms was carried out by the researchers of the lab and professional divers. Three groups of marine invertebrates have predominated in all our collections: ascidians, soft corals, and sponges.

For each sample, one part is usually sent to biologists for identification and the other part is immediately frozen and kept at -20°C until processed.

2.3 Strategy and Approach of the Research

In order to isolate and purify the secondary metabolites, the frozen tissue of the sample is usually cut up and extracted at ambient temperature by organic solvents such as methanol, chloroform or methylene chloride. After filtration and solvent evaporation under reduced pressure, the crude material obtained is then fractionated by chromatographic techniques such as thin layer chromatography, liquid chromatography, middle pressure liquid chromatography or high pressure liquid chromatography. Once the crude extract is obtained, three different strategies for drug discovery can be applied: chemically driven, biologically driven, and a combination of both.

In the *chemically driven* approach, which has been pursued mainly by academic research groups, the objective of the search was to find novel compounds from marine sources. Hence, crude extracts are screened by thin layer chromatography, liquid chromatography coupled to mass spectrometry, and/or nuclear magnetic resonance for unusual and interesting patterns. The next step for this approach is then finding biological properties for purified compounds.

The *biologically driven* strategy is the bioassay-guided approach. This method which involves screening crude extracts for biological activity, followed by the crucial work of backtracking the active compounds from the “hit”-extracts dominates natural products research up to the present. However, this method required considerable effort to get access to sufficient quantities of raw material for reproduction, isolation, structure elucidation, and subsequent verification of biological activity. The complete process proved to be highly time and capacity consuming.

The *combination of the chemically and biologically driven approaches* means that selecting extracts for chemical fractionation is based on the biological activity profile of the crude extract. However, instead of using a bioassay-guided approach to purify the compounds responsible for the activity of the extract, nuclear magnetic

resonance and some chromatographic techniques are used to isolate the chemically most interesting substances. Ideally the structurally unusual or novel compounds are also responsible for the activity of the extract.

The strategy adopted by the research team of the LCSNSA is a combination of the chemically and biologically driven approaches; the research being mainly focused on the discovery of molecules with antitumor, antiviral, antimalarial, antioxidant, and antifouling activities.

2.4 Overview of the Results

More than 2000 samples were chemically and biologically investigated during the past two decades. A total of 32 marine invertebrates have retained our attention for their chemical composition: 18 sponges, 8 ascidians, 4 soft corals, and 2 gorgonians. All contained new compounds, including alkaloids, terpenoids, sterols, lipids, macrolides, cyclic peptides, fatty acid esters, and polyethers, some of which display interesting bioactivity. This paper aims to provide representative examples of our discoveries.

2.4.1 *Luffariella cf. variabilis*

Luffariella variabilis is a coral reef sponge which is widely distributed through the Indo-Pacific, in particular in Palau, where it is estimated to be one of the 10 most common sponges in the reef around the islands.

Luffariella cf. variabilis



Luffariella variabilis is known to produce a bioactive sesterterpene called manoalide. This compound was first reported as an antimicrobial agent but its most important activity was as an analgesic and anti-inflammatory agent. Since the discovery of manoalide in 1980 by De Silva and Scheuer [3], marine sponges of the genus *Luffariella* and more generally of the family thorectidae have proved to be a rich source of bioactive manoalide-related compounds. More than 60 natural manoalide derivatives have been described during the last three decades and most of them were reported to be potent anti-inflammatory agents [4].

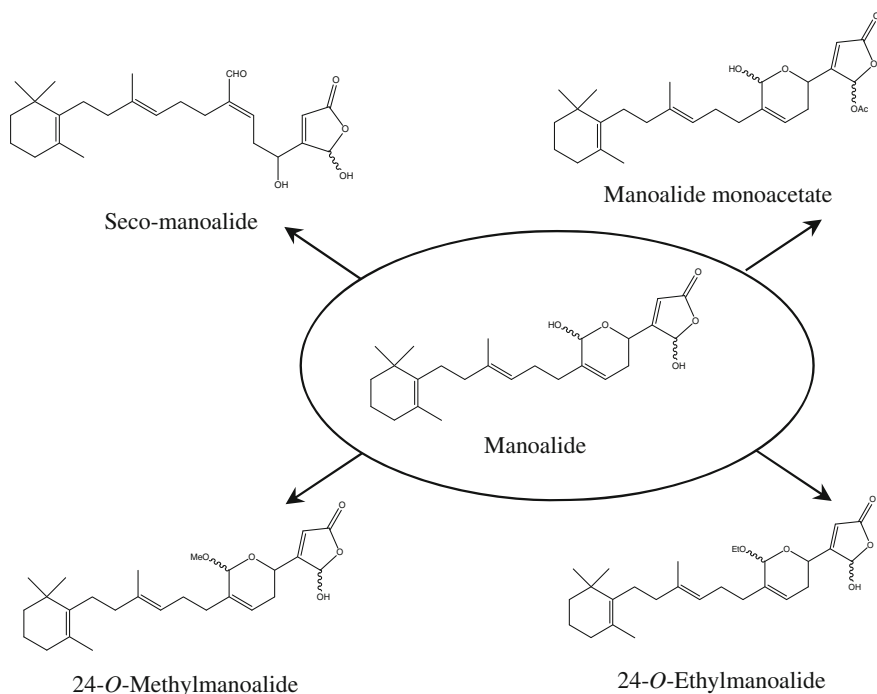


Fig. 2.1 Manoalide and manoalide-related sesterterpenes isolated from *Luffariella cf. variabilis* (Mayotte)

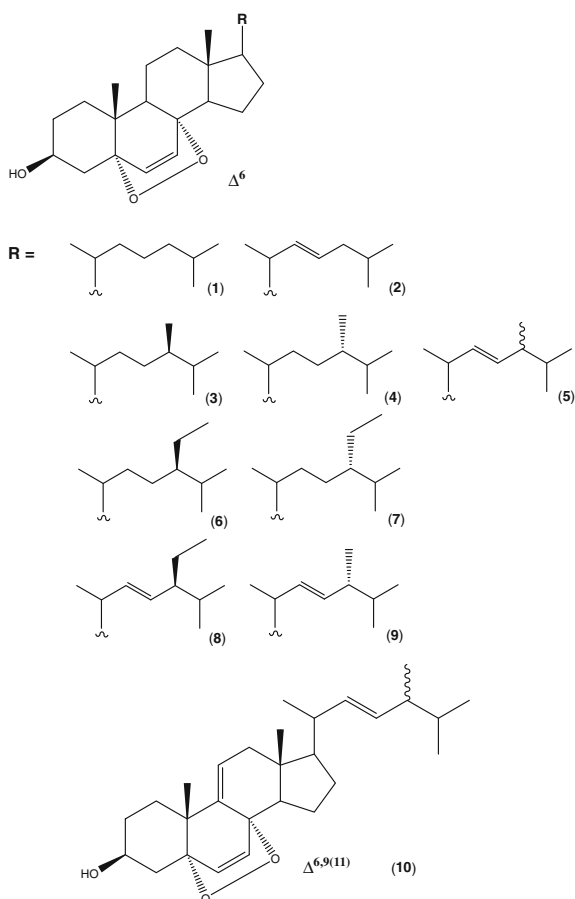
In our effort to discover and develop new bioactive marine natural products, we investigated *L. variabilis*, collected off the coast of Mayotte. Analysis of the secondary metabolites from this sponge resulted not only in the isolation of manoalide and some of its derivatives (Fig. 2.1) [5] but also of 10 bioactive unusual steroids (Fig. 2.2) [6].

This group of steroids with oxygenated functionalities in the nucleus constitutes a long-recognized class of metabolites isolated from marine organisms such as sponges, ascidians, sea hares, gorgonians, sea anemones, mussels, and other marine specimens. However, this was the first time they were isolated from a sponge of the genus *Luffariella*, and that attention is called to their potential antiviral and anti-tumor activities.

Since the crude lipidic extract of *L. variabilis* was found in a preliminary test to be toxic for the brine shrimp *Artemia salina*, the mixture of the 10 steroids was submitted to a series of antiviral and antitumoral assays. The metabolites displayed a significant and selective activity against the human T-cell leukemia-lymphotropic virus type I. The reduction until 50 % of β -galactosidase whose production is directly related to HTLV-I activity, was observed for a concentration of 0.3 $\mu\text{g/mL}$.

These steroids were assayed on the human immunodeficiency virus (HIV). However, no inhibitory activity was observed. Finally, the mixture was screened for

Fig. 2.2 Epidioxysterols isolated from *Luffariella cf. variabilis* (Mayotte)



in vitro cytotoxicity against the human breast cancer cell line and 87 % inhibition was observed at a concentration of 80 $\mu\text{g/mL}$. Hence, it appears obvious that other assays are now needed to precisely ascertain the antiviral and antitumor activities of individual epidioxysterol isolated in useful quantities.

2.4.2 *Fascaplysinopsis* sp.

Again, with the aim of searching for new bioactive molecules, a Madagascan sponge of the genus *Fascaplysinopsis* was investigated. This sponge was collected four times (in Salary Bay at around 100 km north of Tulear) on the south west coast of Madagascar.

Fascaplysinopsis sp.

The chemical investigations of this sponge led to the isolation of 16 unusual compounds (Fig. 2.3): 13 novel nitrogenous macrolides designated as Salarins A-J and Tulearins A-C; 2 new closely related lipodepsipeptides, Taumycins A and B; and 1 nitrogenous bismacrolide, Tausalarin C [7–13].

As the crude lipidic extract of the sponge was found to be toxic for the brine shrimp *A. salina*, the pure molecules were tested for their cytotoxicity against two different human leukemic cell lines (K562 and UT-7). While salarins A, C, D, E, H, J, tulearin A, taumycine A, tausalarin C displayed dose- and time-dependent inhibition of proliferation, salarins F, G, I, and taumycin B were not active in these assays (Table 2.1). Salarin B as well as tulearins B and C were not tested.

2.4.3 *Didemnum molle*

Ascidians have also been shown to be a rich source of cyclic peptides. *Didemnum molle*, a small, 1–5 cm in height, white-greenish, vase-like ascidian, is quite common in deep water of many reefs. This ascidian is green inside due to symbiotic prokaryotic unicellular algae, a symbiosis which might be responsible for the differences of secondary metabolites obtained from animals collected in divergent locations.

Didemnum molle

From two localities in the lagoon of Mayotte and two localities in the lagoon of Tulear in Madagascar, six cyclic hexapeptides were isolated [14, 15]: comoramides A and B, mayotamides A and B, and didmolamides A and B (Fig. 2.4). As similar

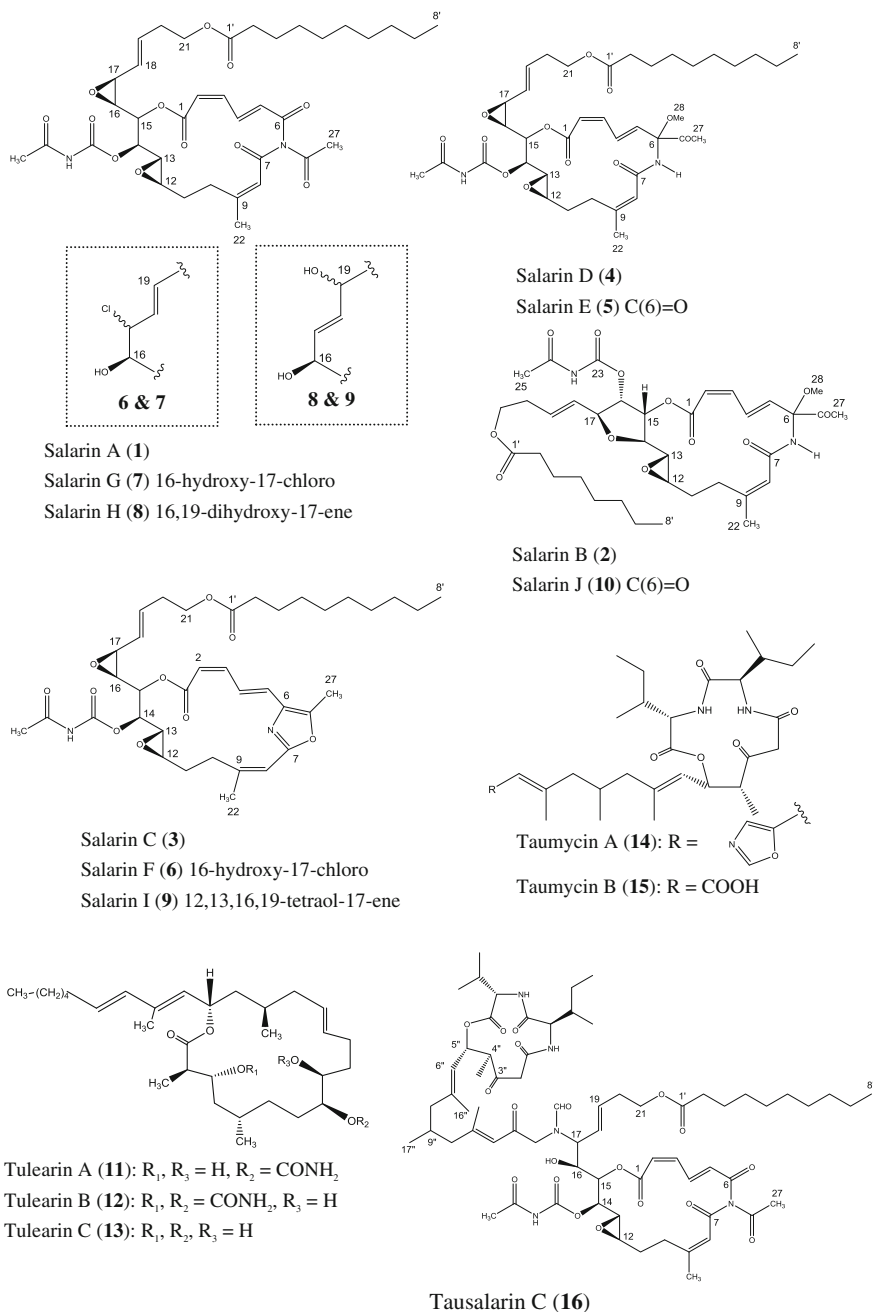


Fig. 2.3 Macrolides isolated from *Fascaplysinopsis* sp. (Madagascar)

Table 2.1 Inhibition of cell proliferation of human leukemic cell lines K562 and UT-7 among the macrolides isolated from *Fascaplysinopsis* sp. (Madagascar)

Compounds	Concentration (μM)	K562*	Concentration (μM)	UT-7*
Salarin A (1)	No activity detected		1	20 % (72 h)
Salarin B (2)	Not tested			
Salarin C (3)	0.1	50 % (24 h)	1	50 % (24 h)
Salarin D (4)	1	30–50 % (72 h)	No activity detected	
Salarin E (5)	1	30–50 % (72 h)	1	60 % (72 h)
Salarin F (6)	No activity detected			
Salarin G (7)	No activity detected			
Salarin H (8)	1	30–50 % (72 h)	No activity detected	
Salarin I (9)	No activity detected			
Salarin J (10)	1	30–50 % (72 h)	No activity detected	
Tulearin A (11)	1	60 % (72 h)	1	35 % (72 h)
Tulearin B (12)	Not tested			
Tulearin C (13)	Not tested			
Taumycin A (14)	No activity detected		1	50 % (24 h)
Taumycin B (15)	No activity detected			
Tausalarin C (16)	1	74 % (72 h)	No activity detected	

*% inhibition of cell proliferation for 24 or 72 h

cyclic hexapeptides have been isolated from cyanobacteria, it can be suggested that the cyclic hexapeptides in *D. molle* also originate from a cyanobacterium.

Moreover, the various cyclic peptides isolated from *D. molle* were screened against several cultured tumor cell lines (A549, HT29, and MEL-28) and were shown to be mildly cytotoxic with a half maximal inhibitory concentration range from 5 to 10 μ g/mL (IC_{50} = 5–10 μ g/mL).

2.4.4 *Biemna laboutei*

The Poecilosclerid sponge *Biemna laboutei* from Madagascar was also investigated for its chemical composition. This sponge was collected several times on the southeast coast of Madagascar: near Sainte-Marie Island (2004), at Itampule (2005), at Salary Bay (2009), and Dos de la Baleine (2011).

The crude extract of the sponge was found to have cytotoxic, anti-plasmodium, and anti-oxidant activities.

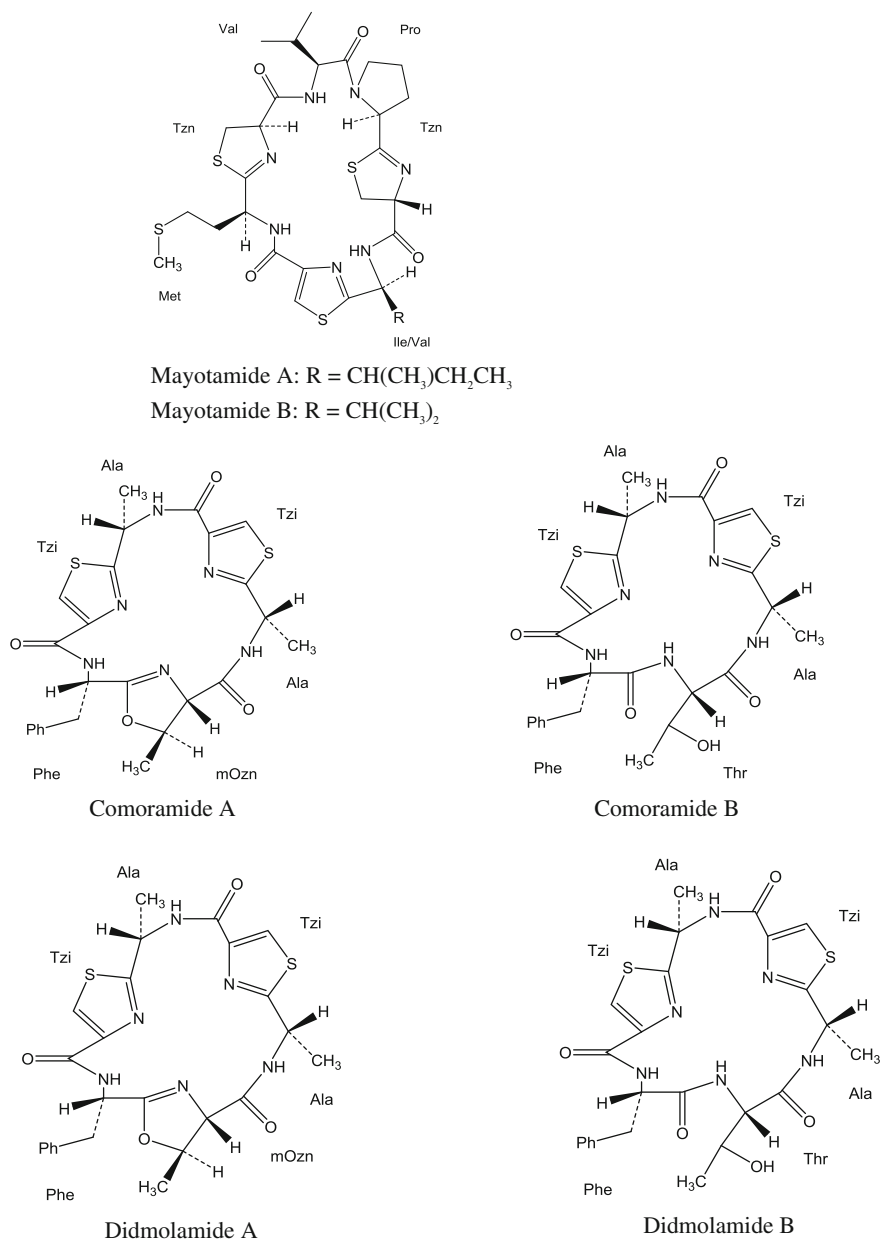


Fig. 2.4 Cyclic hexapeptides isolated from *Didemnum molle* (Mayotte)



Biemna laboutei

Our chemical investigation resulted in the isolation of 25 guanidine alkaloids, among which 19, designated netamines A-S, were new compounds [16–18]. These tricyclic alkaloids can be grouped on the basis of unsaturation and double bond positions to pyrimidines, $\Delta^{8,8a}$ -, $\Delta^{8a,8b}$ - or saturated (5,6,8b)-triazaperhydroacenaphthylene skeletons (Figs. 2.5, 2.6, 2.7 and 2.8).

As for the bioactivity of the isolated molecules, netamines C and D were found to be particularly cytotoxic against lung (A549), colon (HT29), and breast (MDS-MB-231) cancer cells with half maximal inhibitory concentration values in the micromolar range; netamines N, O, Q and particularly M were found to be

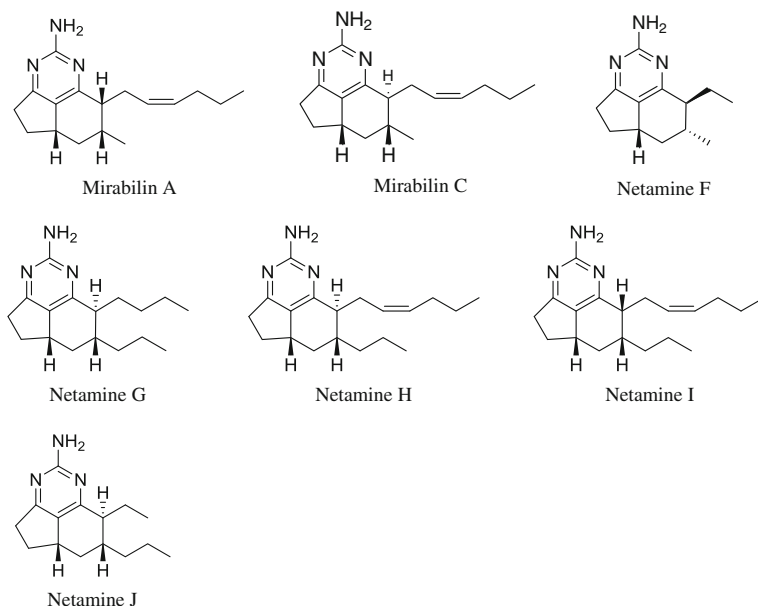


Fig. 2.5 Tricyclic alkaloids with a pyrimidine (5,6,8b)-triazaperhydroacenaphthylene skeleton isolated from *Biemna laboutei* (Madagascar)

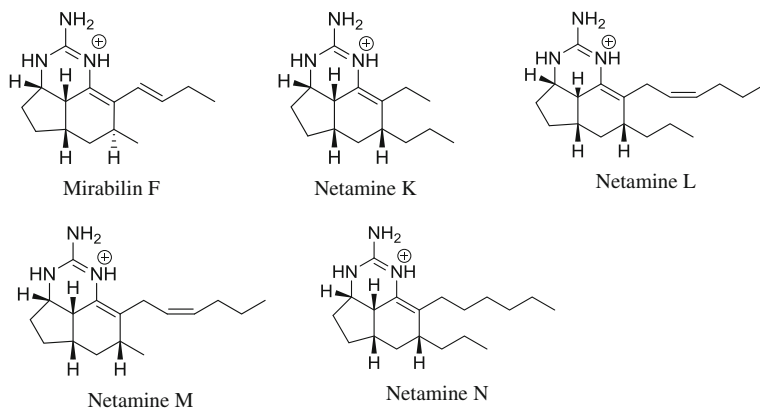


Fig. 2.6 Tricyclic alkaloids with a $\Delta^{8,8a}$ -(5,6,8b)-triazaperhydroacenaphthylene skeleton isolated from *Biemna laboutei* (Madagascar)

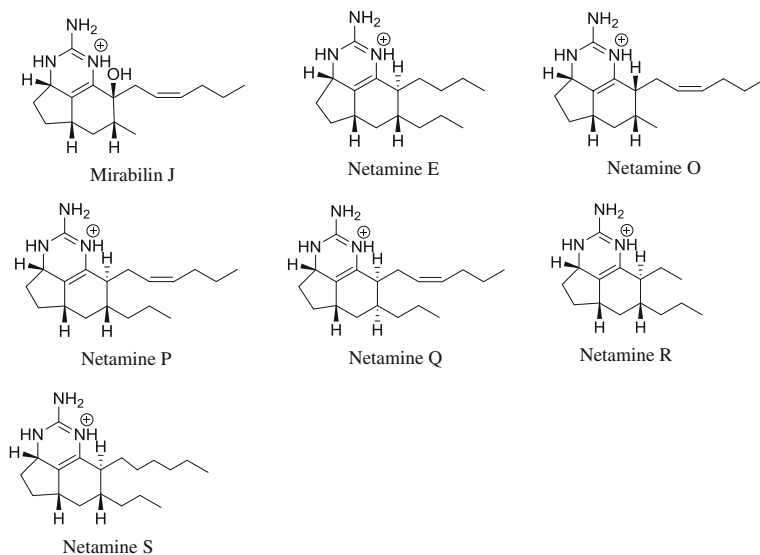


Fig. 2.7 Tricyclic alkaloids with a $\Delta^{8a,8b}$ -(5,6,8b)-triazaperhydroacenaphthylene skeleton isolated from *Biemna laboutei* (Madagascar)

cytotoxic against the KB cell line, and to finish, netamines O, Q and mainly K exhibited antimalarial activity evaluated through in vitro anti-plasmodium activity.

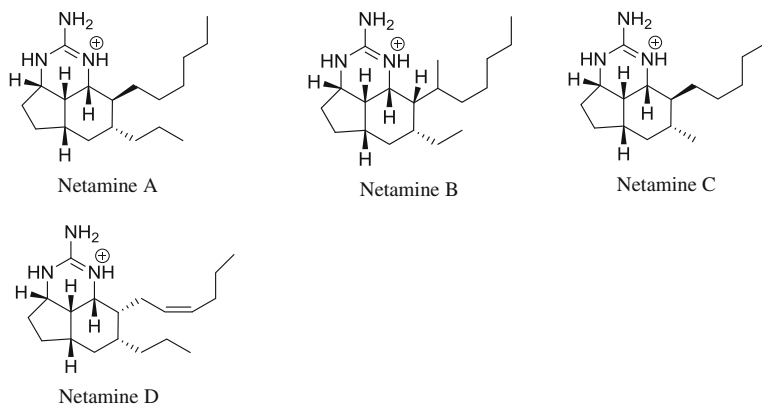


Fig. 2.8 Tricyclic alkaloids with a saturated-(5,6,8b)-triazaperhydroacenaphthylene skeleton isolated from *Biemna laboutei* (Madagascar)

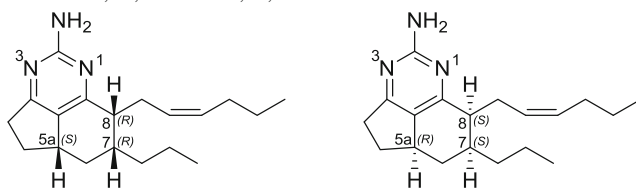
2.5 What are the Absolute Configurations of Molecules Isolated from *Biemna Laboutei* Sponge?

2.5.1 Introduction

We take an interest in determination of the absolute configuration of netamines I and J (Fig. 2.9) which are pyrimidine derivatives.

Why investigate absolute configuration of natural products? Once the structure of the molecule is determined by NMR, we do not still know its absolute configuration. Indeed, NMR allows differentiating diastereoisomers, but gives identical

Netamine I: 5a*S*,7*R*,8*R* or 5a*R*,7*S*,8*S* enantiomers



Netamine J: 5a*S*,7*R*,8*S* or 5a*R*,7*S*,8*R* enantiomers

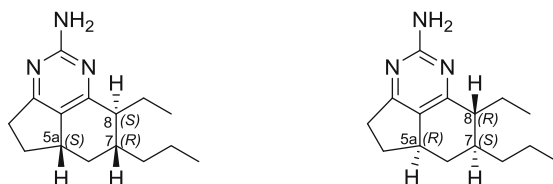
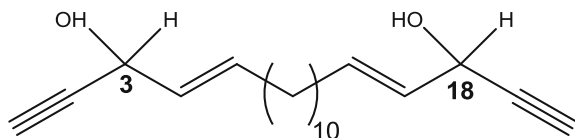


Fig. 2.9 Enantiomers of netamines I and J

Fig. 2.10 Diol isolated from a marine sponge of the genus *Callyspongia*



signals for enantiomers. A pair of enantiomers has identical physical and chemical properties (chemical shifts, NOESY correlations, UV-Vis, or IR spectrum, ...) unless these enantiomers interact with another chiral entity or polarized light.

Moreover, drugs work by interaction with the body's enzymes and receptors. Some form of chiral selectivity is thus expected. Therefore, a pair of enantiomers might have different biological activities. For example, both enantiomers of (4*E*,16*E*)-icosa-4,16-diene-1,19-diyne-3,18-diol (Fig. 2.10) were isolated from a marine sponge of the genus *Callyspongia* [19]. The inhibitory effect on the proliferation of rat lymphatic endothelial cells of enantiomer (–)-3*R*,18*R* was higher than that of enantiomer (+)-3*S*,18*S*.

One of the possibilities to determine an absolute configuration is to carry out X-ray structure. However, all compounds do not crystallize. Thus, their X-ray structures and therefore their absolute configuration cannot be obtained. So, for netamines I and J isolated in oil form, another method was preferred and undertaken: a joint experimental and theoretical electronic circular dichroism (CD) study.

In natural products chemistry, the investigated compounds are often available only in low quantities. Therefore, electronic CD, requiring small quantities [less than one mg (0.1–1 mg)], is usually the most appropriate method.

CD is based on the fact that left and right circular polarized light is absorbed differently by chiral molecules. Therefore, two different extinction coefficients can be observed, ϵ_L and ϵ_R . The difference $\Delta\epsilon = \epsilon_L - \epsilon_R$ is plotted versus wavelengths. Because this difference can become positive or negative, the absorption bands in a CD spectrum can also exhibit different signs. Compared to conventional UV/visible spectroscopy, this is an additional, useful spectral “dimension”, since it makes the CD technique more sensitive to geometric and electronic properties of the analyzed molecule. As a pair of enantiomers have opposite CD spectra, comparison between experimental and quantum chemically calculated CD spectra might allow assigning the absolute configuration of a molecule. However, sophisticated computational methods that simultaneously yield accurate excitation energies, band intensities, and signs must be used.

2.5.2 Experimental and Computational Details

UV and electronic CD spectra were recorded in methanol solution: UV spectra on a Varian Cary 100 Scan spectrometer and electronic CD spectra were obtained using a Jasco J-810 spectropolarimeter.

All calculations were performed using Gaussian 09 program [20]. The SMD continuum model [21] was used to simulate solvent effect on ground state geometry and excited state energies and properties. ω B97XD density functional [22] was used to compute ground state and vertical excited states (TD-DFT). This functional is a long-range corrected hybrid density functional with damped atom–atom dispersion corrections [23] and includes an increasing fraction of exact exchange when the interelectronic distance increases. This functional significantly improves the description of charge-transfer excited states and was also chosen as it well reproduced the topology of experimental UV peaks though transition energies were significantly overshoot [24, 25]. Two Pople's style basis sets were used for ground state (6-31+G(d,p) and excited states (6-31++G(d,p)). An additional set of diffuse functions was added in TD-DFT to improve the band shape of UV spectrum. The 30 lowest singlet transition energies were computed.

Six main stages for the calculation of electronic CD spectrum can be described:

1. The chiroptical behavior of a chiral compound depends on the spatial orientation of its chromophoric groups and thus on its molecular flexibility. Therefore, electronic CD spectrum is very conformation-dependent. Consequently, in many cases, it is not sufficient to simply consider the global minimum but it is also necessary to take into account the electronic CD contributions of all conformational species that are significantly populated at ambient temperature. This, in turn, requires a detailed conformational investigation beforehand. According to Boltzmann statistics, all minimum conformers that are significantly populated at 25 °C have relative free energies lower than 3 kcal/mol.
2. These energetically favorable minimum structures are then subjected to the calculations of excited states.
3. In this step, the Boltzmann-weighted UV spectrum is shifted in order to obtain the same wavelength as in experimental UV spectrum for the first absorption band. In fact, TD- ω B97XD method yields too large transition energies in most cases [24]. The goal of the UV-shift is to cancel this error in theoretical UV spectrum. The same UV-shift is then used in theoretical electronic CD.
4. In the simulation of electronic CD, the decisive quantity is the rotatory strength. The computed rotatory strengths are transformed into units of $\Delta\epsilon$ and superimposed with Gaussian functions centered at the respective wavenumbers of the electronic transitions. The same exponential half-width value ($\Delta\sigma = 0.2$ eV) as for UV spectrum was used.
5. All electronic CD spectra are weighted via Boltzmann statistics at 298.15 K in order to obtain the total spectrum.
6. In the last step, experimental and Boltzmann-weighted electronic CD spectra are compared. Wavelengths of positive and negative Cotton effects are used to assign the absolute configuration.

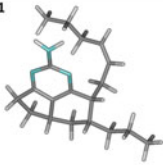

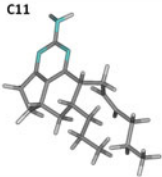
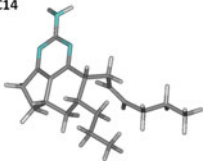
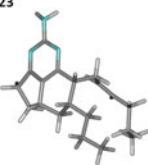
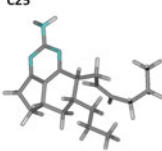
2.5.3 Results for Netamine I

The process described above was applied to netamine I. Seventeen conformers were optimized with a relative free energy lower than 3 kcal/mol. Among them, six have a population $\geq 5\%$ (Table 2.2). The main differences between conformer's geometries arise from positions of the two side-chains.

Calculated UV spectra of the 17 conformers are shown in Fig. 2.11. These UV spectra are rather similar. The graph located on the right side displays the Boltzmann-weighted UV spectrum which is UV-shifted by -0.583 eV. The UV-shift is calculated to fit the first experimental UV absorption band at 304 nm (bottom right side of Fig. 2.11). Second and third calculated absorption band wavelengths are also in better agreement with experimental UV spectrum (resp. 234 and 202 nm). However, the calculated relative intensity of the third band (at 197 nm) is higher than that of the second band, whereas it is the opposite in the experimental UV spectrum. The first and the second absorption bands are, respectively, associated with the intensity of a single transition, whereas the third band results from the addition of the intensities of several transitions. That might be the reason the relative calculated intensity of the third band is wrong.

The theoretical CD spectra of the 17 conformers associated to the 5*aS*,7*R*,8*R* configuration of netamine I are shown in Fig. 2.12. The UV-shift (-0.583 eV) was applied for all conformers. These spectra are rather different because electronic CD spectrum is very conformation-dependent. Therefore, it is important to get an average spectrum via Boltzmann statistics (Fig. 2.12b).

Table 2.2 Netamine I conformers having a population $\geq 5\%$ and their relative free energies

					
ΔG (kcal/mol)	1.26	ΔG (kcal/mol)	1.23	ΔG (kcal/mol)	0
Population	5 %	Population	5 %	Population	42 %
					
ΔG (kcal/mol)	0.93	ΔG (kcal/mol)	1.03	ΔG (kcal/mol)	0.58
Population	9 %	Population	7 %	Population	16 %

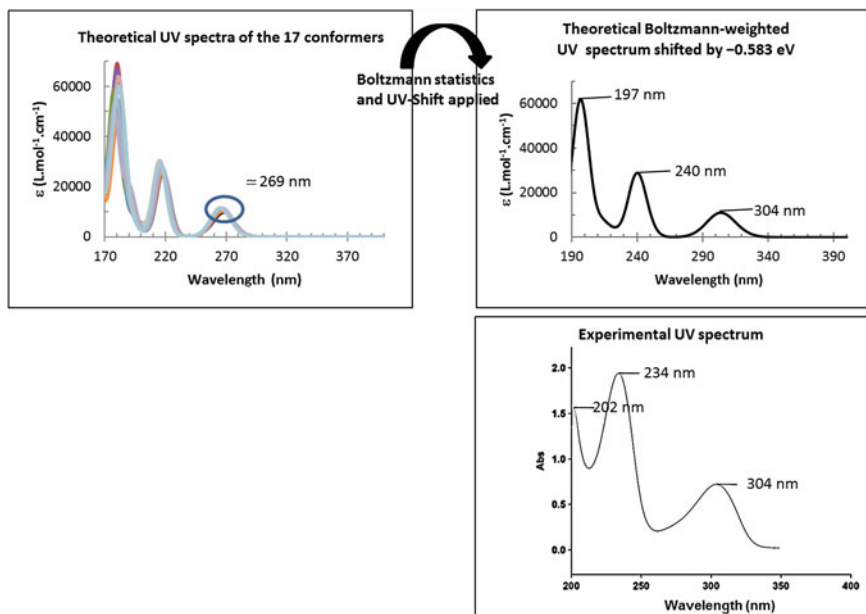


Fig. 2.11 Theoretical and experimental UV spectra of netamine I

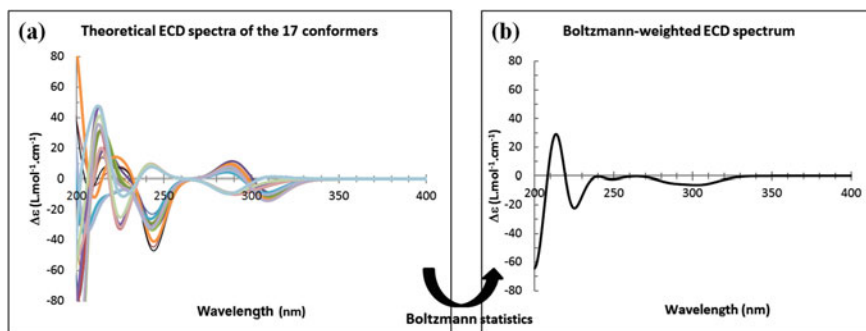


Fig. 2.12 Theoretical electronic CD spectra of the 5a*S*,7*R*,8*R* configuration of netamine I

The comparison of experimental and theoretical electronic CD spectra (Fig. 2.13) suggests that 5a*S*,7*R*,8*R* enantiomer was isolated from *Biemna laboutei* sponge because there is only one positive Cotton effect around 210 nm (calc. 214 nm), and after 220 nm, $\Delta\epsilon$ is always negative in calculated and experimental spectra for this enantiomer.

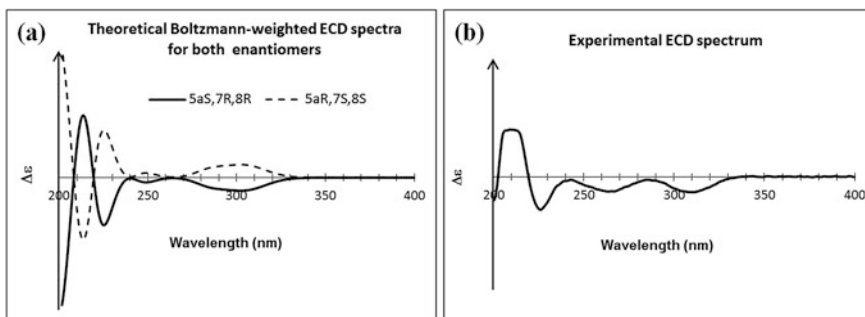


Fig. 2.13 Theoretical CD spectra (a) and experimental CD spectrum (b) of netamine I

2.5.4 Results for Netamine J

The same process was applied to netamine J. A total of 29 conformers with relative free energies lower than 3 kcal/mol were optimized. The Boltzmann-weighted UV spectrum (Fig. 2.14) was UV-shifted by -0.594 eV to achieve a better fit to experimental band wavelengths. As for netamine I, the calculated relative intensity of the third band is too high compared to experimental UV spectrum.

The comparison of experimental and theoretical electronic CD spectra (Fig. 2.15) suggests that the 5a*S*,7*R*,8*S* enantiomer was isolated from *Biemna laboutei*, despite the agreement to experiment is worse than for netamine I. Indeed, the experimental positive Cotton effect at 243 nm was not found in the calculated spectra of the 5a*S*,7*R*,8*S* enantiomer. Therefore, another density functional (B3LYP) was used to get a better understanding of this default. A total of 22 conformers with relative free energies lower than 3 kcal/mol were optimized at B3LYP/6-31+G(d,p) level. Then UV and electronic CD spectra were computed at TD-B3LYP/6-31++G(d,p) level using both B3LYP and ω B97XD geometries. Both

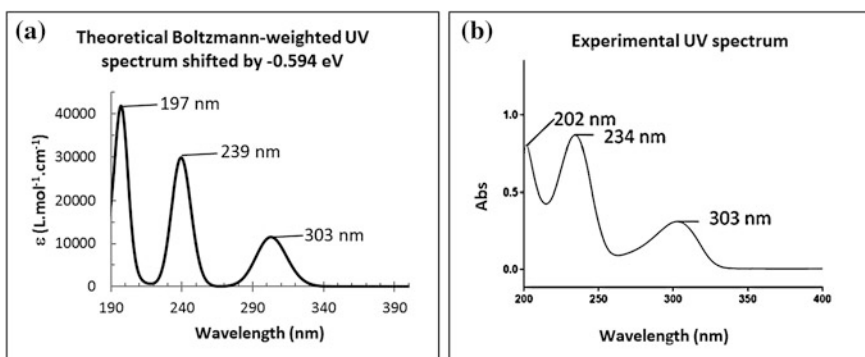


Fig. 2.14 Theoretical (a) and experimental (b) UV spectra of netamine J

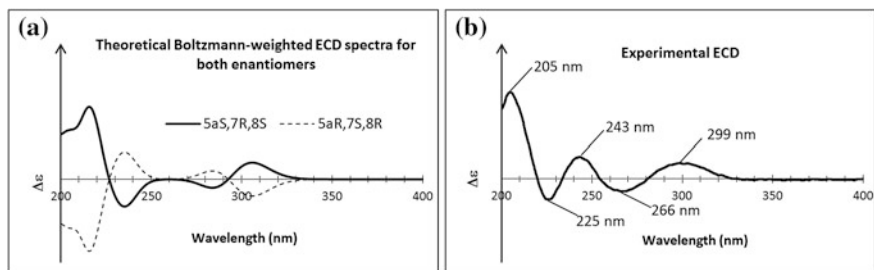


Fig. 2.15 Theoretical electronic CD spectra of both enantiomers (a) and experimental electronic CD spectrum (b) of netamine J

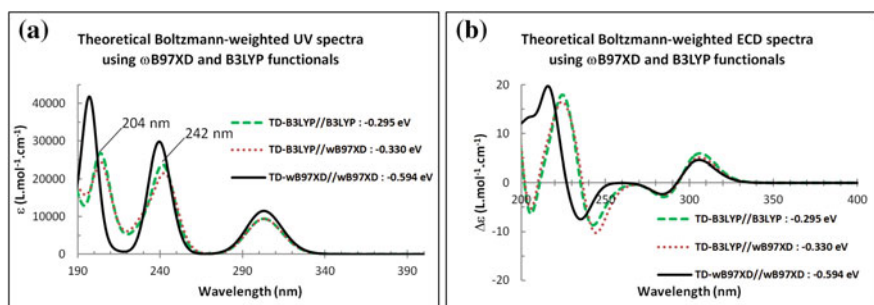


Fig. 2.16 Theoretical UV (a) and electronic CD spectra (b) of 5aS,7R,8S enantiomer of netamine J calculated using ωB97XD and B3LYP functional

Table 2.3 Experimental and calculated Cotton effect wavelengths of netamine J

$\lambda_{\text{Exp.}}$ (nm)	CE*	$\lambda_{\text{Calc.}} - \lambda_{\text{Exp.}}$ (nm)		
		TD-B3LYP//B3LYP	TD-B3LYP//ωB97XD	TD-ωB97XD//ωB97XD
299	(+)	+7	+7	+7
266	(-)	+19	+19	+18
243	(+)	—	—	—
225	(-)	+18	+20	+10
205	(+)	+19	+19	+11

* positive (+) or negative (-) Cotton effects

methods are respectively indicated by TD-B3LYP//B3LYP and TD-B3LYP//ωB97XD in Fig. 2.16 and Table 2.3.

The comparison between TD-B3LYP and TD-ωB97XD UV spectra (Fig. 2.16) shows that intensities of the three UV bands are smaller at the TD-B3LYP level. TD-B3LYP relative intensity of the third band (at 204 nm) is always higher than the intensity of the second band but to a lesser extent than that occurring with TD-ωB97XD. The UV-shifts (−0.295 and −0.330 eV) applied to TD-B3LYP spectra to fit the first experimental absorption wavelength are smaller than TD-ωB97XD

one's. TD-B3LYP position of the third absorption band wavelength (204 nm; TD- ω B97XD: 197 nm) is in better agreement to experiment (202 nm) but this is the reverse for the second band (TD-B3LYP: 242, TD- ω B97XD: 239, exp.: 234 nm).

TD-B3LYP spectra (UV and CD) calculated using B3LYP and ω B97XD geometries, are rather similar.

The comparison between TD-B3LYP, TD- ω B97XD (Fig. 2.16b) and experimental CD (Fig. 2.15b) spectra and their Cotton effect wavelengths (Table 2.3) clearly show that (1) the UV shift applied to CD spectra seems to be slightly overestimated for all Cotton effect wavelengths calculated for all methods (e.g. +7 nm for the first Cotton effect), (2) TD- ω B97XD performs slightly better than TD-B3LYP, particularly under 220 nm, (3) none of the methods is able to reproduce the experimental positive Cotton effect at 243 nm for the 5a*S*,7*R*,8*S* enantiomer.

2.5.5 Perspectives

In conclusion, absolute configurations of netamines I and J were determined. Absolute configurations of 17 other netamines have to be determined.

The question is how to improve agreement between experimental and calculated electronic CD spectra in the future? (1) More elaborate time-dependent density functionals might be used as those of Stefan Grimm B2PLYP [26]. This functional combines a standard linear response TD-DFT treatment of a hybrid density functional with a configuration interaction singles with perturbative doubles correction. (2) A DFT/Multireference Configuration Interaction method might also be used to take into account the non dynamic electron correlation [27]. (3) Vibronic coupling might also be taken into account to improve the band shapes of electronic CD spectrum [28]. This last task will require geometry optimization of several excited states, which might be quite time consuming.

2.6 Conclusion

To conclude, the marine environment of the Indian Ocean's western part remains as one of the exceptional and largest untapped reservoirs for marine invertebrate taxonomy and the discovery of novel bioactive compounds. The LCSNSA located in the heart of Indian Ocean's western part is clearly at a strategic place for the marine natural products research in this area. The work presented in this paper is just an illustration of the long-term project on marine natural products exploitation by the LCSNSA. This project is still at the very beginning and there are numerous areas that have not been investigated by the LCSNSA, particularly in the microbial environment. Indeed, there is increasing evidence that many metabolites are not

produced by the animals themselves but by associated bacterial symbionts. In the future, the expansion of our research to biotechnology will be one of our key strategic challenges.

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