BRAZILIAN JOURNAL OF OCEANOGRAPHY, 58(3):213-218, 2010

CHEMICAL DEFENSES OF THE TROPICAL MARINE SEAWEED *Canistrocarpus cervicornis* AGAINST HERBIVORY BY SEA URCHIN

*Éverson Miguel Bianco[1], Valéria Laneuville Teixeira[2] and Renato Crespo Pereira[2]**

[1]Instituto de Química, Universidade Federal Fluminense
Pós-Graduação em Química Orgânica
(Morro do Valonguinho, s/n, 24020-150 Niterói, RJ, Brasil)

[2]Instituto de Biologia, Universidade Federal Fluminense
Departamento de Biologia Marinha
(P.O. Box 100.644, CEP 24001-970, Niterói, RJ, Brasil)

*Corresponding author: renato.pereira@pq.cnpq.br.

ABSTRACT

This paper reports on the defensive chemical properties of the marine tropical brown seaweed *Canistrocarpus cervicornis* against herbivory. A natural concentration of dichloromethane crude extract (DCE) obtained from this seaweed significantly inhibited feeding by the sea urchin *Lytechinus variegatus*. The major metabolite isolated from this active DCE extract was identified as the (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14-hydroxydolast-1(15),8-diene that strongly inhibited feeding by the same sea urchin. This result suggests that the dolastane diterpenes class may constitute the defensive system of *C. cervicornis* against herbivory, and probably also of that of other brown seaweeds endowed with a biosynthetic pathway capable of producing compounds of the dolastane-type, a typical skeleton found in Dyctioteae species worldwide. This is the first report showing this compound-type (dolastane diterpenes) as a chemical defense against herbivory in marine seaweeds. This study constitutes an additional report broadening the known spectrum of action and roles of secondary metabolites of the *C. cervicornis* and Dyctioteae species.

RESUMO

Este artigo demonstra a química defensiva anti-herbivoria da macroalga parda marinha *Canistrocarpus cervicornis*. Em sua concentração natural, o extrato bruto em diclorometano (DCE) inibiu significativamente o consumo alimentar do ouriço-do-mar *Lytechinus variegatus*. Deste extrato em DCE foi isolado o metabólito majoritário identificado como o diterpeno (4*R*,7*R*,14*S*)-4α,7α-diacetoxi-14-hidroxidolasta-1(15),8-dieno. Esses resultados comprovam que diterpenos da classe dolastano podem compor o sistema defensivo anti-herbivoria de *C. cervircornis* e, supostamente, o de outras algas pardas capazes de produzi-los, uma vez que são composto típicos encontrados em esqueletos de Dyctiotas em todo o mundo. Este é o primeiro estudo demonstrando a ação anti-herbivoria desta classe de metabolitos (diterpenos dolastanos) em macroalgas marinhas. Tais informações ampliam o conhecimento sobre o papel desses metabolitos especiais em *C. cervicornis* e de espécies de Dyctiotae.

Descriptors: *Canistrocarpus cervicornis*, Chemical defenses, Dolastanes, Diterpenes.
Descritores: *Canistrocarpus cervicornis*, Defesas químicas, Dolastanos, Diterpenos.

INTRODUCTION

Marine seaweeds produce a wide variety of secondary metabolites such as terpenes, sterols, polyphenols, acetogenins and others (BLUNT et al., 2010). Several recent studies have revealed that some of these compounds function as chemical defenses able to deter a broad range of natural enemies including competitors, epiphytes, pathogenic bacteria and herbivores (AMSLER; FAIRHEAD, 2006; PAUL et al., , 2006; PEREIRA; DA GAMA, 2008). Several of these herbivores such as fishes, sea urchins, crabs and gastropods affect seaweed communities and their dynamics profoundly (JOHN et al., 1992), because large quantities of seaweed biomass can be consumed, especially in tropical environments. To avoid, minimize, or tolerate the damage caused by herbivory, seaweeds exhibit several kinds of adaptation to reduce their attractiveness, and chemical defenses are one of the most conspicuous strategies manifested by tropical seaweeds (PAUL et al., 2001; PEREIRA; DA GAMA, 2008).

In tropical and warm-temperate seas, brown seaweeds (e.g. *Dictyota*, *Dictyopteris*, and others) produce complex mixtures of terpenoids, and terpenoid-aromatics originated by mixed biosynthesis, besides polyphenols and acetogenins (HAY; FENICAL, 1988; PAUL, 1992). Many of these secondary metabolites are known to play several ecological roles, the diterpenes including the most studied compound types of the *Dictyota* species. Currently over 300 diterpenes are known, from at least 35 species of *Dictyota* collected all around the world (VALLIM et al., 2005).

There are many examples of diterpenes from brown seaweeds acting as chemical defenses, mainly the similar diterpenes from the so-called dictyol structural family, such as dictyol E, dictyol B, dictyol B acetate, dictyol H, all of which have been shown to be active anti-feeding metabolites (HAY; STEINBERG, 1992; SCHMITT et al., 1995; PEREIRA et al., 2000b). Pachydictyol A, found in several species of *Dictyota*, inhibits herbivory by tropical parrotfish species, the temperate fishes *Lagodon rhomboides* (Linnaeus) and *Diplobus holbrooki* (Bean), and the sea urchin *Diadema antillarum* (Philippi). Dictyol E reduces herbivory by *L. rhomboides*, *D. holbrooki* and the sea urchin *Arbacia punctulata* (Lamarck). Pachydictyol A, dictyol B and dictyol H inhibit herbivory in the rabbitfish *Siganus doliatus* (Guérin-Méneville) (HAY; STEINBERG, 1992).

Very few distinct diterpenes of the dictyol structural class are known to exhibit defensive properties. For example, the compound (6*R*)-6-hydroxydichotoma-3,14-diene-1,17-dial from *Dictyota menstrualis* (Hoyt) Schnetter, Hörnig et Weber-Peukert, inhibits herbivory by the amphipod *Parhyale hawaiensis* (Dana) (PEREIRA et al., 2000a), and the diterpene of the dolabellane class, the 10-18-diacetoxy-8-hidroxy-2,6-dolabelladiene from *Dictyota pfaffii* Schnetter, inhibits herbivory by the sea urchin *Lytechinus variegatus* (Lamarck) (BARBOSA et al., 2004).

Moreover, there are other diterpene structural classes found in *Dictyota* species, such as dolastanes and *seco*-dolastanes isolated from *Dictyota cervicornis* Kützing and *Dictyota crispata* Lamoroux (TEIXEIRA et al., 1986a, b; KELECOM; TEIXEIRA, 1988; DE PAULA et al., 2007; VALLIM et al., 2005), but little is known of the ecological role of these diterpenes, excepting the mixture of the *seco*-dolastane diterpenes linearol/isolinearol as defense against the gastropod *Astraea latispina* (Philippi) (PEREIRA et al., 2002). Continuing our ecological studies of the defensive chemicals of Dictyotales (PEREIRA et al., 1994, 2000a, b, 2002; BARBOSA et al., 2004), the present study shows the action of a new class of defensive compounds in Dictyotales, a dolastane diterpene isolated from *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck, which acts as a feeding deterrent against the sea urchin *Lytechinus variegatus*. *Canistrocarpus cervicornis* (previously identified as *Dictyota cervicornis*, DE CLERCK et al., 2006) is a brown marine alga of the Dictyotaceae family that also contains bicyclic and tricyclic diterpenes as major metabolites (BIANCO et al., 2009).

MATERIAL AND METHODS

Organisms

Specimens of the brown seaweed *Canistrocarpus cervicornis* (Dictyoteae, Ocrophyta) were collected in the Ribeira Bay, Angra dos Reis City, Rio de Janeiro State (23:00:34 S, 44:26:10 W), Brazil, in August 2004, at depths between 1 and 4 m. After collection, the algal material was immediately cleaned of epiphytic organisms and air dried. Voucher specimens were deposited in the herbarium at the Universidade do Estado do Rio de Janeiro, Brazil (HRJ 10772). Specimens of the sea urchin *Lytechinus variegatus* were collected on Itaipu Beach, Niterói City, Rio de Janeiro State for the feeding assays.

Preparation of the Crude Extracts

The air-dried algal material (900 g) was extracted in CH_2Cl_2 at room temperature, yielding 44 g of dichloromethane crude extract (DCE). This algal material was extracted in EtOAc using similar procedures to yield 2.8 g of ethyl acetate crude extract (EACE), and finally, after extraction with MeOH, yielded 0.9 g of methanol crude extract (MCE).

Isolation and Identification of the Major Compound

The DCE crude extract was subjected to silica gel 230-400 mesh column chromatography (7 cm x 10 cm) eluted with *n*-hexane, $CHCl_3$, AcOEt, Me_2CO and MeOH. Part of the fraction in $CHCl_3$ (7.5 g) was fractionated by silica gel 70-230 mesh column chromatography (3 cm x 40 cm) eluted with a *n*-Hex/AcOEt mixture (9:1) to give 72 fractions of 30 mL each. Fractions 17-57 (1.8 g) were reunited after TLC analyses and purified by silica gel 230-400 mesh column chromatography (2 cm x 34 cm) eluted with a *n*-Hex/AcOEt mixture (3:1), yielding 450 mg of pure (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14-hydroxydolast-1(15),8-diene (Fig. 1), as colorless gum.

Next, the DCE (dichloromethane crude extract) was assayed by ^{1}H NMR and the spectra shifts showed a dolastane diterpene as the major compound: 4.91 (bs, 1H), 4.86 (bs, 1H), 0.88 (s, 3H), 2.79 (septet, 1H, J = 6.9), 0.94 (d, 3H, J = 6.9), 0.91 (d, 3H, J =

6.9), 1.47 (s, 3H), 2.15 (s, 3H), 2.02 (s, 3H), 3.71 (bs, 1H) (GONZÁLEZ et al., 1983; SUN et al., 1981). The complete structural elucidation of this diterpene was obtained by ^{1}H NMR and ^{13}C-APT NMR assays, the spectral data having been previously reported (GARCIA et al., 2009).

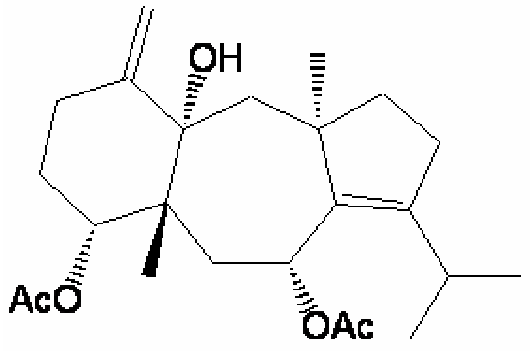

Fig. 1. Major diterpene compound isolated from *C. cervicornis*.

Feeding Deterrence Assay

The defensive properties of the extracts in dichloromethane (DCE), ethyl acetate (EACE), and methanol (MCE) from *C. cervicornis* were evaluated using artificial food wafers prepared from freeze-dried green alga *Ulva fasciata* and agar (HAY et al., 1994), and tested on the sea urchin *L. variegatus*, an ecologically-relevant consumer (SOUZA et al., 2008).

Natural concentrations of the extracts were produced to correspond to the content found in 2g of *C. cervicornis*, the major compound corresponding to 0.81% (dry weight of this seaweed), according to the literature (the authors, not published). The controls were prepared by adding 0.90 g of agar to 20.0 mL of distilled water warmed in a microwave oven until the agar was liquefied. This mixture was added to 16.0 mL of cold water containing 2.0 g of the freeze-dried powder of *U. fasciata* and CH_2Cl_2. The treatments (containing either the extract or the pure compound) were prepared by similar procedures, but the chemicals were previously dissolved in CH_2Cl_2 for further incorporation of 2.0 g of freeze-dried powder of *U. fasciata*. Next, the organic solvent was removed by rotatory evaporation. This procedure was necessary to obtain a uniform coating of natural products on the algal particles before the addition of the agar.

Controls and treatments were hardened in a mold screen and cut into small pieces (10 x 10 squares with 1.5 mm sides), thus finishing preparation of the food wafers which were then offered simultaneously to the sea urchin *L. variegatus*.

The feeding deterrence assays were carried out in a 500 L aquarium with circulating seawater. The sea urchins were conditioned separately in small plastic containers of 0.5 L each, but together with control and treated food wafers.

The assays were monitored each 12 h, until a clear feeding preference for control or treatment wafers was observed. The defensive properties of the crude extract and of the pure compound were then estimated by comparing the number of squares consumed, of either control or treated experimental food wafers.

Statistical Analyses

The Wilcoxon matched pairs test, a nonparametric test equivalent to a paired *t*-test, was used to evaluate the statistical significance of the results obtained. Treatments and controls were considered significant when $p < 0.05$ ($\alpha = 5\%$).

RESULTS

The DCE, the most non-polar extract, was very effective in inhibiting feeding by the sea urchin *L. variegatus* (p = 0.001; n = 22). In contrast, EACE showed no defensive activity against the same consumer (p = 0.1531; n = 25), whereas MCE, the most polar extract, stimulated consumption by *L. variegatus* (p = 0.0175; n = 22) when compared to the respective controls (Fig. 2).

From the most consumed deterrent extract (DCE) against *L. variegatus* was isolated the major diterpene compound identified as (4*R*,7*R*,14*S*)-4α,7α-Diacetoxy-14-hydroxydolast-1(15),8-diene (GARCIA et al., 2009).

In complementary assays, we showed that this diterpenoid significantly inhibited feeding by the urchin *L. variegatus* (p = 0.0143; n = 31) (Fig. 3).

DISCUSSION

Several species of *Dictyota* have been studied extensively and are known to produce a broad spectrum of chemical compounds that act as feeding deterrents against herbivores, or exhibit other biological functions (HAY; STEINBERG, 1992; PEREIRA; DA GAMA, 2008). However, most known deterrents are structurally similar diterpenes called dictyols, a structural class of the prenylated guaiane diterpenes found in several *Dictyota* species (TEIXEIRA; KELECOM, 1987). In contrast to the species producing dictyols, *Canistricarpus cervicornis* (previously *Dictyota cervicornis*) is susceptible to fish grazing (LITTLER et al., 1983; LEWIS, 1986), does not produce dictyol-type compounds (KELECOM; TEIXEIRA, 1988), and was almost never used for domicile construction by the amphipod *Pseudoamhpitoides incurvaria* just as protection against predation (HAY et al., 1990). In this study we ascertained that *C. cervcornis* may be chemically defended against herbivory, precisely as other species of *Dictyota* which also produce dolastanes (similar chemical structures), but not by producing dictyols as do *D. divaricata* Lamouroux, *D. linearis* (C. Agardh) Greville, *D. indica* Sonder ex Kützing *and D. furcellata* (C. Agardh) Greville.

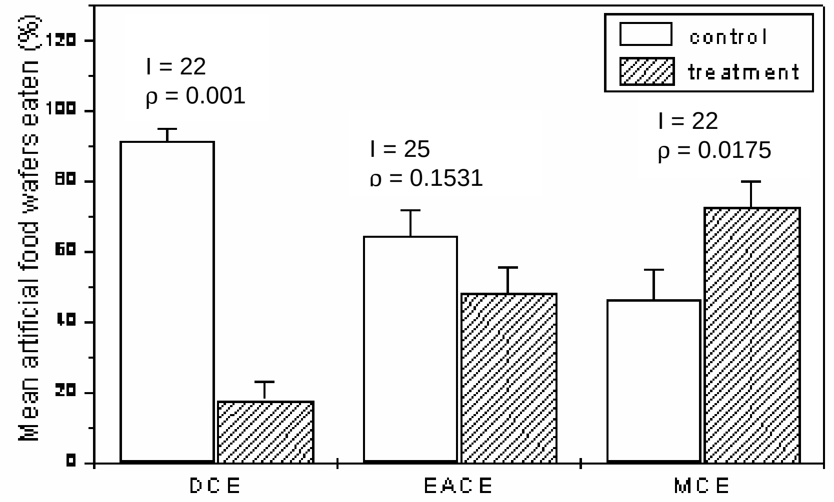

Fig. 2. Effect of the natural concentrations of dichloromethane crude extract (DCE), ethyl acetate crude extract (EACE), and methanol crude extract (MCE), obtained from *C. cervicornis* on feeding by the sea urchin *L. variegatus*. Vertical bars in each histogram represent mean food mass eaten + SE. Significant differences at $p < 0.05$ (Wilcoxon test).

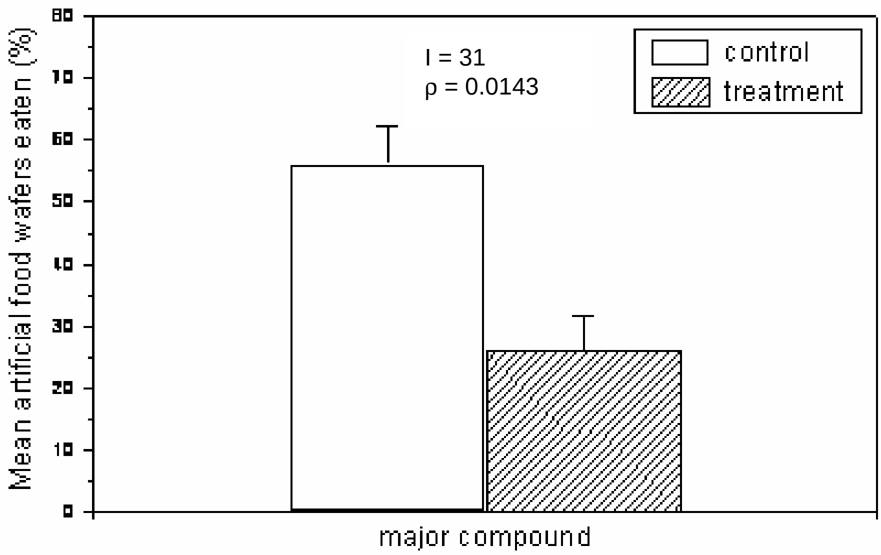

Fig. 3. Effect of the natural concentration (0.81% – dry weight) of major compound found in *C. cervicornis* on feeding by the sea urchin *L. variegatus*. Vertical bars in each histogram represent mean food mass eaten + SE. Significant differences at $p < 0.05$ (Wilcoxon test).

The different results regarding the susceptibility of *Dictyota* species to consumers may be due to some other reason than just the structural class of chemicals produced by them.

Recent studies on the Rocas Atoll, Brazil, showed that the brown alga *C. cervicornis*, a great dolastane-class diterpenes producer, was not eaten by herbivores as little as species producing dictyol-class diterpenes (MARQUES et al., 2006). A similar situation was also observed on a Caribbean barrier reef, where *C. cervicornis* suffered exceptionally low losses to fish grazing (LITTLER et al., 1983). Another study showed that several crude extracts of this macroalga exhibited ichthyotoxic properties against the fish *Carassius auratus*, probably due to the same chemical defenses (DE LARA-IASSI et al., 2000). This study confirms the strong feeding deterrence due to the non-polar dichloromethane extract and the pure major (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14-hydroxydolast-1(15),8-diene obtained from *C. cervicornis* against the sea urchin *L. variegatus*. These results indicate that dolastane class diterpenes may play a significant role as chemical defenses against herbivores, similar to that of the dictyol diterpenes class. However, we suppose that other compounds from this alga could also act as deterrents against this sea-urchin, since the magnitude of this activity is much greater for the crude extract (~ 80% relative to controls) than for the pure compound (~ 50% relative to controls), even though both were tested at approximately natural concentrations.

In fact, the defensive chemicals produced by *Dictyota* species may be due to compounds other than dictyol types. Four other types of dictyotalean metabolite skeletons are today known to act as chemical defenses against herbivores, including the diterpenoid (6*R*)-hydroxydichotoma-3,14-diene-1,17-dial, a dichotomane diterpene skeleton type found in *Dictyota menstrualis* (Hoyt) Schnetter, Horning et Weber-Peukert (PEREIRA et al., 2000a). The dictyodial compound (xeniane skeleton type), a probable biogenetic precursor of the diterpene (6*R*)-hydroxydichotoma-3,14-diene-1,17-dial (TEIXEIRA; KELECOM, 1987), is another exception. A xeniane compound also found in *D. menstrualis* may deter feeding by sea urchins, but not by pinfish or amphipods (CRONIN; HAY, 1996). The third exception is a mixture containing isolinearol/linearol compounds – both constituted by skeletons of the secodolastane type (PEREIRA et al., 2002), and the fourth is the dolabellane compound 10,18-diacetoxy-8-hydroxy-2,6-dolabelladiene, found as the major compound in *Dictyota pfaffii* Schnetter (BARBOSA et al., 2004).

If geographical diterpene distribution patterns may be considered within an evolutionary perspective, rather than as a purely ecological topic (BARBOSA et al., 2004), the defensive action of dolastane diterpene in *Dictyota* (here *Canistrocarpus*) species adds a new component to enable us to understand the complex world of the evolution of defensive chemistry in seaweeds. In fact, more conclusive ecological and/or evolutionary approaches will only be possible when more information from different oceans worldwide becomes available.

ACKNOWLEDGEMENTS

We wish to thank the National Brazilian Research Council (CNPq) for a PhD fellowship (EMB) and for a research productivity fellowship (RCP and VLT). We are grateful to Dr. Maria Tereza M. Széchy and Dr Joel Campos De Paula for their help in the collection and identification of the seaweed studied. Comments by two reviewers were invaluable in improving the manuscripts and are gratefully acknowledged.

REFERENCES

AMSLER, C. D.; FAIRHEAD, V. A. Defensive and sensory chemical ecology of brown algae. **Adv. Bot. Res.**, v. 43, p. 1-91, 2006.

BARBOSA, J. P.; TEIXEIRA, V. L.; PEREIRA, R. C. A dolabellane diterpene from the brown alga *Dictyota pfaffii* as chemical defense against herbivores. **Bot. Mar.**, v. 47, p. 147-151, 2004.

BIANCO, E.M.; ROGERS, R.; TEIXEIRA V.L.; PEREIRA, R.C. Antifoulant diterpenes produced by the brown seaweed *Canistrocarpus cervicornis*. **J. Appl. Phycol.**, v. 21, p. 341–346, 2009.

BLUNT J.W.; COPP B.R.; MUNRO, M.H.G.; NORTHCOTE, P.T.; PRINSEP, M.R. Marine natural products. **Nat. Prod. Rep.**, v. 27, p. 165-237, 2010.

CRONIN, G.; HAY, M. E. Chemical defenses, protein content, and susceptibility to herbivory of diploid vs. haploid stages of the isomorphic brown alga *Dictyota ciliolata* (Phaeophyta). **Bot. Mar.**, v. 39, p. 395-399, 1996.

CRONIN, G.; PAUL, V. J.; HAY, M. E.; FENICAL, W. Are tropical herbivores more resistant than temperate herbivores to seaweed chemical defenses? Diterpenoid metabolites from *Dictyota acutiloba* as feeding deterrents for tropical versus temperate fishes and urchins. **J. Chem. Ecol.**, v. 23, p. 289-302, 1997.

DE CLERCK, O.; LELIAERT, F.; VERBRUGGEN, H.; LANE, C. E.; DE PAULA, J. C.; PAYO, D. I.; COPPEJANS, E. A revised classification of the Dictyoteae (Dictyotales, Phaeophyceae) based on rbcL and 26S ribosomal DNA sequence data analyses. **J. Phycol.**, v. 42, p. 1271-1288, 2006.

DE LARA-IASSI, G.; ÁLVAREZ-HERNÁNDEZ, S.; COLLADO-VIDES, L. Ichthyotoxic activity of extracts from Mexican marine macroalgae. **J. Appl. Phycol.**, v. 12, p. 45-52, 2000.

DE PAULA, J. C.; CASSANO, V.; YONESHIGUE-VALENTIN, Y.; TEIXEIRA, V. L. Diterpenes from the Brazilian brown alga *Dictyota crispata* (Dictyotaceae, Phaeophyta). **Nat. Prod. Comm.**, v. 2, p. 135-137, 2007.

GARCIA, D. G.; BIANCO, E. M.; SANTOS, M. C. B.; PREIRA, R. C.; FARIA, M. V. C.; TEIXEIRA, V. L.; BURTH, P. Inhibition of Mammal $Na^{+}K^{+}$-ATPase by diterpenes extracted from the Brazilian brown alga *Dictyota cervicornis*. **Phytother. Res.**, v. 23, p. 943-947, 2009.

GONZÁLEZ, A. G.; MARTIN, J. D.; NORTE, M.; RIVERA, P.; PERALES, A.; FAYOS, J. F. Structure and absolute configurations of *Dictyota* sp. diterpenes. **Tetrahedron**, v. 39, p. 3355-3357, 1983.

HAY, M. E.; DUFFY, J. E.; PFISTER, C. A.; FENICAL, W. Chemical defense against different marine herbivores: are amphipods insects equivalents? **Ecology**, v. 68, p. 1567-1580, 1987a.

HAY, M. E.; FENICAL, W.; GUSTAFSON, K. Chemical defense against diverse coral-reef herbivores. **Ecology**, v. 68, p. 1581-1591, 1987b.

HAY, M. E.; FENICAL, W. Marine plant-herbivore interactions: the ecology of chemical defense. **Ann. Rev. Ecol. Syst.**, v. 19, p. 111-145, 1988.

HAY, M. E.; DUFFY, J. E.; FENICAL, W. Seaweed chemical defenses: among compound and among-herbivore variance. **Int. Coral Reef Symp.**, 6., Australia, 1988. v. 3, 43-48, 1988a.

HAY, M. E.; RENAUD, P. E.; FENICAL, W. Large mobile versus small sedentary herbivores and their resistance to seaweed chemical defenses. **Oecologia**, v. 75, p. 246-252, 1988b.

HAY, M. E.; DUFFY, J. E.; FENICAL, W. Host-plant specialization decreases predation on a marine amphipod: an herbivore in plant's clothing. **Ecology**, v. 71, p. 733-743, 1990.

HAY, M. E.; STEINBERG, P. D. The chemical ecology of plant-herbivore interactions in marine versus terrestrial communities. In: ROSENTHAL, G. A.; BERENBAUM, M. R. (Ed.) **Herbivores: their interactions with secondary plant metabolites**. Academic Press, San Diego, California, USA., v 2, p. 371-413, 1992.

HAY, M. E.; KAPPEL, Q. E.; FENICAL, W. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification and plant quality. **Ecology**, v. 75, p. 1714-1726, 1994.

JOHN, D. M.; HAWKINS, S. J.; PRICE, J. H. **Plant-animal interactions in the marine benthos**. Clarendon Press, Oxford, England, 1992.

KELECOM, A.; TEIXEIRA, V. L. Dolastane diterpenes from the marine brown alga *Dictyota cervicornis*. **Phytochemistry**, v. 27, p. 2907-2909, 1988.

LEWIS, S. M. The role of herbivorous fishes in the organization of a Caribbean reef community. **Ecol. Monogr.**, v. 56, p. 183-200, 1986.

LITTLER, M. M.; TAYLOR, P. R.; LITTLER, D. S. Algal resistance to herbivory on a Caribbean barrier reef. **Coral Reefs**, v. 2, p.111-118, 1983.

MARQUES, L. V.; VILLAÇA, R.; PEREIRA, R. C. Susceptibility of macroalgae to herbivorous fishes at Rocas Atoll, Brazil. **Bot. Mar.**, 49: 379-385, 2006.

PAUL, V. J. **Ecological roles of marine natural products**, 1st ed. New York: Comstock Publishing Associates - Cornell University Press, 1992.

PAUL, V. J.; CRUZ-RIVERA, E.; THACKER, R. W. Chemical mediation of macroalgal herbivore interactions: ecological and evolutionary perspectives. In: MCCLINTOCK, J. B.; BAKER, B. J. (Ed.). **Marine chemical ecology**. Boca Raton, Fla.: CRC Press, 2001. p. 227-265, 2001.

PAUL, V. J.; PUGLISI, M. P.; RITSON-WILLIAMS, R. Marine chemical ecology. **Nat. Prod. Rep.**, v. 23, p. 153-180, 2006.

PEREIRA, R. C.; TEIXEIRA, V. L.; KELECOM, A. Chemical defenses against herbivores in marine algae. 1. The brown alga *Dictyota dichotoma* (Hudson) Lamouroux from Brazil. **An. Acad. Bras. Ci.**, v. 66, p. 229-235, 1994.

PEREIRA, R. C.; CAVALCANTI, D. N.; TEIXEIRA, V. L. Effects of secondary metabolites from the tropical Brazilian brown alga *Dictyota menstrualis* on the amphipod *Parhyale hawaiensis*. **Mar. Ecol. Prog. Ser.**, v. 205, p. 95-100, 2000a.

PEREIRA, R. C.; DONATO, R.; TEIXEIRA, V. L.; CAVALCANTI, D. N. Chemotaxis and chemical defenses in seaweed susceptibility to herbivory. **Rev. Bras. Biol.**, v. 60, p. 405-414, 2000b.

PEREIRA, R. C.; PINHEIRO, M. D.; TEIXEIRA, V. L.; DA GAMA, B. A. P. Feeding preferences of the endemic gastropod *Astraea latispina* in relation to chemical defenses of Brazilian tropical seaweeds. **Braz. J. Biol.**, v. 62, p. 33-40, 2002.

PEREIRA, R. C.; DA GAMA, B. A. P. Macroalgal chemical defenses and their role in structuring tropical marine communities. In: AMSLER, C. D. (Ed.). **Algal Chemical Ecology**. New York: Springer, p. 25-55, 2008.

SCHMITT, T. M.; HAY, M. E.; LINDQUIST, N. Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. **Ecology**, v. 76, p. 107-123, 1995.

SOUZA, C. F.; OLIVEIRA, A. S.; PEREIRA, R. C. Feeding preference of the sea urchin *Lytechinus variagatus* (Lamarck, 1816) on seaweeds. **Braz. J. Oceanogr.**, v. 56, p. 239-247, 2008.

SUN, H. H.; MCCONNELL, O. J.; FENICAL, W.; HIROTSU, K.; CLARDY, J. Tricyclic diterpenoids of the dolastane ring system from the marine alga *Dictyota divaricata*. **Tetrahedron**, v. 37, p. 1237-1242, 1981.

TEIXEIRA, V. L.; KELECOM, A. Geographic distribution of the diterpenes from the marine brown alga *Dictyota* Lamouroux (Dictyotales, Phaeophyta). **Nerítica**, v. 2, p. 179-200, 1987.

TEIXEIRA, V. L.; TOMASSINI, T.; FLEURY, B. G.; KELECOM, A. Dolastane and secodolastane diterpenes from the marine brown alga *Dictyota cervicornis*. **J. Nat. Prod.**, v. 49, p. 570-575, 1986a.

TEIXEIRA, V. L.; TOMASSINI, T.; KELECOM, A. Cervicol, a further secodolastane diterpene from the marine brown alga *Dictyota cervicornis* Kützing. **Bull. Soc.Chim. Belg.**, v. 95, p. 263-268, 1986b.

VALLIM, M. A., DE PAULA, J. C., PEREIRA, R. C., TEIXEIRA, V. L. The diterpenes from Dictyotacean marine brown algae in the Tropical Atlantic American region. **Biochem. Syst. Ecol.**, v. 33, p. 1-16, 2005.

(Manuscript received 2 November 2009; revised 25 March 2010; accepted 14 April 2010)