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Diterpenes from marine brown alga *Dictyota guineensis* (Dictyotaceae, Phaeophyceae)

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Abstract: The crude extract of the marine brown alga *Dictyota guineensis* was analyzed by high-resolution gas chromatography-mass spectrometry (HRGC-MS). Five diterpenes were identified: dictyol E (the most abundant diterpene), dictyotadiol, dictyoxide, isopachydictyol A and pachydictyol A, all diterpenes from the chemical group I, *i.e.*, mainly prenylated derivatives of known sesquiterpene skeletons that result from a first cyclization of geranyl-geraniol between positions 1 and 10. These diterpenes are known for their activity against bacteria, fungi and other activities. The results characterize *D. guineensis* as a species that yields exclusively diterpenes from group I, with low oxidation and low structural complexity. On Brazilian coasts, only *D. mertensii* provides exclusively prenylated guaiane diterpenes. Although *D. guineensis* presents alternate branches and fixing by rhizoidal branches, it is easily distinguishable from *D. mertensii* by the much narrower stem, short stature and flabelliform habit of the former species. On the other hand, both species have been characterized as producers of diterpenes of group I, in particular, prenylated guaiane. However, *D. guineensis* has a majority dictyol E in the lipophilic extract, while *D. mertensii* produces more complex prenylated guaianes, like dictyol H.

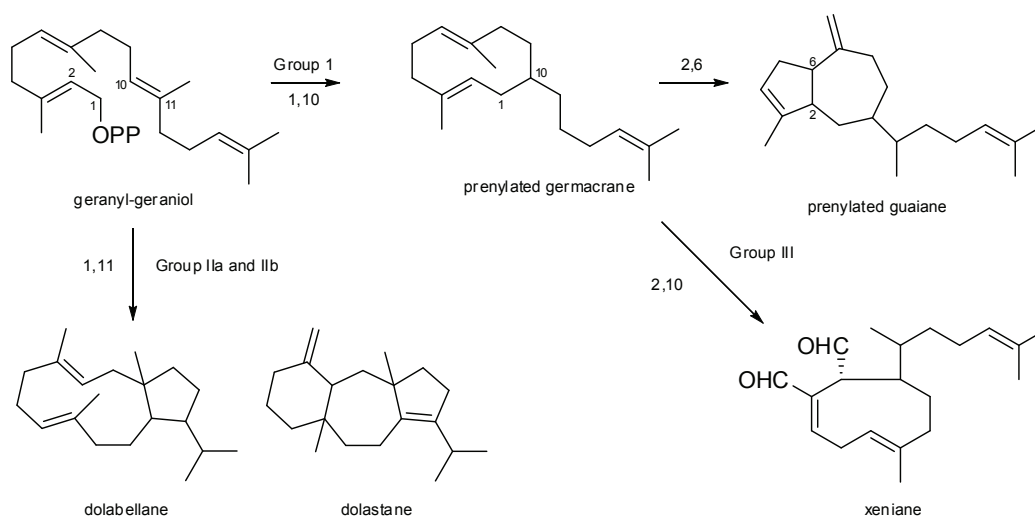
Introduction

The algae of the genus *Dictyota* Lamouroux present difficulties in the establishment of clear limits of separation between species. In previous studies, our group has indicated that the diterpenes from *Dictyota* species may play an important role as taxonomic markers (*e.g.*, De-Paula et al., 2001, 2007a, 2008; Freitas et al., 2007; Teixeira et al., 1990, 2001). On the other hand, the algae of the family Dictyotaceae are very important sources of antiviral natural products (*e.g.*, Pereira et al., 2004, 2005; Barbosa et al., 2004; Cirne-Santos et al., 2006, 2008; Vallim et al., 2010).

Based on a revised biogenetic scheme proposed by Teixeira & Kelecom (1988), the diterpenes have been distributed into three groups (I-III), depending on the structure of the products resulting from the first formal cyclization of the geranyl-geraniol precursor. Thus, group I compounds result from a first cyclization of geranyl-geraniol between positions 1 and 10; group II compounds derive from a first cyclization of the precursor between positions 1 and 11, and group III compounds from either a first formal cyclization between positions 2 and 10 or

by ring contraction of the prenylated germacrane (Scheme 1).

Many species of algae of the genus *Dictyota* have been studied on the Brazilian coast and their chemical composition examined. In many cases, variability is observed between populations along the coast (Teixeira, 2010) and the species were classified into taxonomic groups A, B and C. Taxonomic group A comprises the species of *Dictyota* that produce diterpenes of chemical groups I e III. In this group are included the Brazilian populations of *Dictyota crenulata* J. Agardh (= *Dictyota jamaicensis* W.R.Taylor) (De-Paula et al., 2008), *D. ciliolata* Sonder ex Kützing, and *D. menstrualis* (Hoyt) Schnetter, Hörning & Weber-Peukert (Cavalcanti et al., 2006). *Dictyota mertensii* (Martius) Kützing (Freitas et al., 2007) can be included as a subgroup because, so far, Brazilian and Caribbean populations (like *D. dentata* J.V. Lamouroux) produce only diterpenes of group I, in particular prenylated guaianes (Alvarado & Gerwick, 1985; Freitas et al., 2007). The taxonomic group B includes Brazilian populations of *Dictyota pfaffii* Schnetter (Barbosa et al. 2004) and *Dictyota dolabellana* De Paula, Yoneshigue-Valentin & Teixeira (De-Paula et al.



Scheme 1. Hypothetical biogenetic pathway for “Dictyota” diterpenes of groups I, II (A and B) and III (Teixeira & Kelecom, 1988).

2007b), a new species, both characterized as producing exclusive type dolabellane diterpenes and derivatives. The taxonomic group C comprises the species *Canistrocarpus cervicornis* (Kützing) De-Paula & De Clerck (e.g. Kelecom & Teixeira, 1988, as *Dictyota cervicornis* Kützing) and *C. crispatus* (De-Paula et al., 2007a, as *Dictyota crispata* J.V.Lamouroux), producing exclusively dolastane and secodolastane diterpenes.

Dictyota guineensis (as *Dilophus guineensis* (Kützing) J. Agardh) was cited for the Brazilian coast by Taylor (1930). However, in his review in 1960, Taylor put in doubt the occurrence of this species in Brazil. Only recently, the occurrence of this species in Brazil is cited in the literature (Villaça et al., 2010; Széchy & De Paula, 2010). In the present study, we present the terpenoid composition of the dichloromethane extract from the brown alga *Dictyota guineensis* collected on the Bahia coast. These data may be important for future projects to obtain new prototypes with potential antiviral activity.

Materials and Methods

Algal material

Specimens of *Dictyota guineensis* were collected at Penha Beach, Itamaracá Island, Santa Cruz City, State of Bahia, located in the northeastern part of the country on the Atlantic coast, Brazil, during April, 2004, and January, 2005. The algae were collected at depths ranging from 1 to 2 m. The seaweeds were washed with local sea water and separated from sediments, epiphytes and other associated organisms. The algae were collected and identified by Dr. Joel Campos De Paula and voucher specimens were deposited in the Herbarium of the Universidade do Estado do Rio de

Janeiro and the Museu Nacional, Universidade Federal do Rio de Janeiro (HRJ10.846, HRJ10.312, HRJ10.932, R207.449).

Experimental

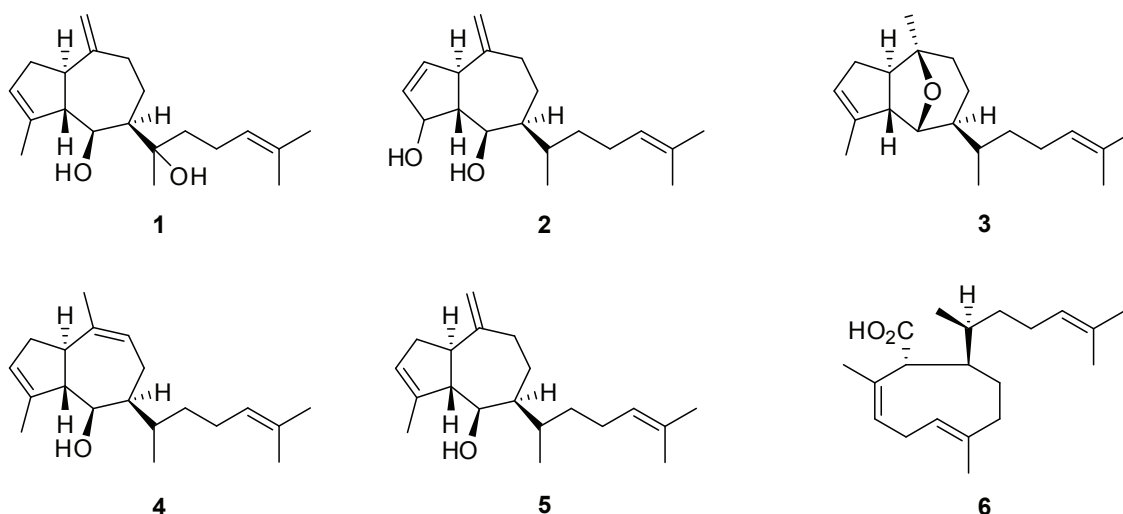
All solvents were HPLC grade. Analytical thin-layer chromatographic (TLC) separations were carried out on Merck silica gel 60 F-254 (0.2 mm) precoated aluminum plates. Once developed, plates were visualized by spraying with 2% ceric sulphate in sulfuric acid, followed by gentle heating. Silica gel 60 (Merck, 70-230 and 230-400 mesh) was used for column chromatography. Nuclear magnetic resonance spectra (NMR) were recorded in CDCl_3 (100% Aldrich) on a Varian Unity Plus 300 spectrometer using TMS as internal standard.

Chemical analysis

Air-dried *D. guineensis* was extracted with dichloromethane (100%) at room temperature. The solvent was evaporated under reduced pressure, yielding brownish residues. An aliquot of the dichloromethane extract was diluted in an appropriate volume of ethyl acetate and analysed by HRGC-MS on a HP 6890 series GC system, coupled to a HP 5973 mass selective detector in the electron impact mode (70 eV), equipped with an HP-1 MS capillary column (30 m x 0.25 mm; film thickness 0.25 μm). Injector and detector temperatures were set at 270 °C and 290 °C, respectively. The temperature was kept at 160 °C, then programmed to 260 °C at a rate of 4 °C/min and finally raised at a rate of 15 °C/min to 290 °C for 15 min. Hydrogen was the carrier gas at a flow rate of

Table 1. Diterpenes detected in two populations of *D. guineensis*.

Diterpenes	Caribbean sea (Puerto Rico)	Brazilian coast (Bahia State)	References
dilophic acid (6)	X		Schlenk & Gerwick (1987)
dictyol E (1)	XX	XX	Schlenk & Gerwick (1987)
present study			
dictyoxide (3)		X	present study
isopachydictyol A (4)		X	present study
pachydictyol A (5)		X	present study
dictyotadiol (2)		X	present study



1 mL/min. Diluted samples were injected manually in the split mode (1/10). The chemical components were identified based on comparisons of their mass spectral data with those of standards and/or literature data, by co-injection of these samples in the HRGC, and from Wiley 275 library data of the HRGC-MS system.

An aliquot (10 mg) of crude extract was dissolved in CDCl_3 and the ^1H NMR (300 MHz) spectrum was obtained in a Varian-Unity Plus 300 (TMS as internal reference).

Results and Discussion

The diterpenes from *Dictyota guineensis* were analyzed by the HRGC-MS technique. This analysis revealed the presence of peaks in the mass spectra compatible with the fragmentation patterns of five diterpenes: dictyol E (the most abundant diterpene), dictyotadiol, dictyoxide, isopachydictyol A and pachydictyol A (1-5). This is the first chemical record for *Dictyota guineensis* from Brazil. The only other study was carried out with algae collected from the coast of Puerto Rico by Schlenk & Gerwick (1987). In this study, two natural products were isolated: dictyol E, a prenylated

guaiane diterpene (1) and the major product, and dilophic acid (6), a xeniane diterpene (Table 1).

In the Brazilian seaweed extract, no compounds of diterpene group III were detected. In the ^1H NMR spectrum, there were no signals that would indicate the presence of aldehydes (present in most of the group III diterpenes) or carboxylic acids (present in dilophic acid). Nevertheless, *D. guineensis* should be considered to belong to the taxonomic group A. Despite the difference in the presence of xeniane diterpenes, the two populations (Puerto Rico and Brazil) presented dictyol E (2) as the major natural product.

The xeniane diterpenes and their derivatives have shown excellent results against HSV-1 (herpes simplex type 1) and HIV-1 (Human immunodeficiency virus type 1) (Ninomya et al., 1995; Pereira et al., 2004, 2005), while prenylated guaianes have not shown promise as candidates for antiviral agents.

For these reasons, studies on the biotechnological potential of the diterpenes of *D. guineensis* as a source of antiviral agents should be discouraged. Moreover, the algae are not abundant and frequent on the Brazilian coast, their populations have a small biomass and the diterpenes can be obtained from more abundant sources or by synthesis

for assessment *in vitro* and *in vivo*.

Nevertheless, the analysis of the chemical composition of *D. guineensis* can be an important tool for taxonomic, phylogenetic and biogeographic studies.

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