10β-Acetoxy-8α,9α-epoxy-14β-hydroxy-7-oxodolastane – A New Diterpene Isolated from the Brazilian Brown Macroalga *Canistrocarpus cervicornis*

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Chemical investigation on the nonpolar extract of the Brazilian brown alga *Canistrocarpus cervicornis* (Dictyotaceae) has led to the isolation of a new diterpene **1** and four known *seco*-dolastane diterpenes **2**–**5**. Their chemical structures were elucidated by 1D- and 2D-NMR spectroscopy, and the data was compared with the literature. A full X-ray diffraction analysis confirmed the absolute configuration of **1**.

Introduction. – In tropical and warm temperate regions, macroalgae of the order Dictyotales (Phaeophyta) are known to produce a wide variety of monocyclic, bicyclic, and tricyclic diterpenoids, such as dolabellane, prenylated guaiane, xeniane, dichotomane, dolastane, and *seco*-dolastane skeleton-types, which exhibit a broad spectrum of biological/ecological activities, including chemical defense mechanisms against herbivores [1], antifouling properties [2–4], antimicrobial [5][6], antiviral [7], cytotoxicity [8], and other activities [9][10].

64% of the known Dictyotacean diterpenes found in the Tropical Atlantic American region belong to dolastane and *seco*-dolastane types [11]. There are only a few diterpenes described so far from other regions [12–14]. Hence, the main goal of this work was to investigate the presence of new compounds, specially diterpenes from this macroalga's family. Among the Dictyotaceae species found on the Brazilian coast, we selected for this study the species *Canistrocarpus cervicornis* (KÜTZING) DE PAULA & DE CLERCK, formerly *Dictyota cervicornis* KÜTZING [15], due to their abundance in the Rio de Janeiro's littoral habitat.

The CH_2Cl_2-soluble part of dried brown alga *C. cervicornis* furnished one new dolastane diterpene **1** and four previously reported *seco*-dolastane diterpenes identified as isolinearol (**2**), isolinearol acetate (**3**), linearol (**4**), and indicol (**5**; *Fig. 1*).

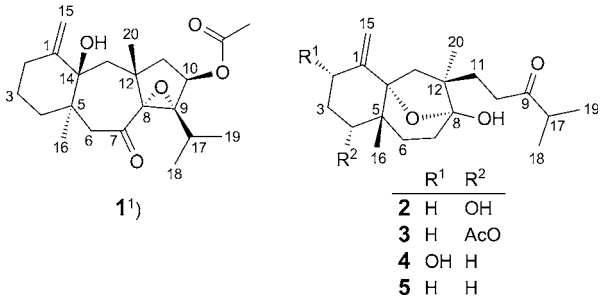


Fig. 1. *Chemical structures of compounds* **1**–**5**

In previous studies, our research group reported many ecological and pharmacological activities from *C. cervicornis*'s diterpenes. They include antifouling [3], feeding deterrent [16], antileishmanial [17], anticoagulant and antiplatelet activities [10], and inhibition of the enzyme Na^+K^+ATPase [9]. These findings suggest that this macroalga could be an important source for natural compounds with pharmacological/biotechnological properties which could be used in the development of new drugs.

Results and Discussion. – One new dolastane diterpene (**1**) and four known *seco*-dolastanes, *i.e.*, isolinearol (**2**), isolinearol acetate (**3**), linearol (**4**), and indicol (**5**), were isolated from the Brazilian brown alga *C. cervicornis* (*Fig. 1*). The chemical structures of compounds **2**–**5** have been characterized by NMR spectroscopic data (^{1}H and ^{13}C), and by comparison with literature data. The structure of compound **1** was investigated by 1D- and 2D-NMR spectroscopy and by single crystal X-ray diffraction analysis.

The spectroscopic data of the compounds **2**–**5** were identical with those in the literature [12][18][19]. In previous investigations we only suggested the presence of traces of **5** by GC/MS technique [20]. Here, we confirm the presence of **5** by isolation and NMR spectroscopic data.

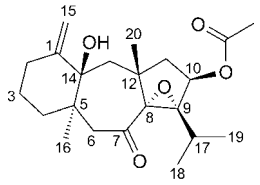
Compound **5** was first isolated from the brown macroalga *Dictyota indica* from the Arabian Sea, together with small amounts of other *seco*-dolastanes, including **2** and **4** [19]. Compound **2** was also previously isolated from *Dictyota linearis* from Japan [18] and *Dictyota cervicornis* from Brazil, together with compounds **3** and **4** [12]. Curiously, there is another diterpene called linearol (with a kaurene skeleton) [21]. This compound was isolated from the superior plant *Sideritis stricta* and doesn't have any relation with **4** (*seco*-dolastane skeleton-type) isolated from the marine macroalga *C. cervicornis*.

Compound **1** was isolated as colorless crystals (1.5 mg) and the molecular formula was deduced as $C_{22}H_{32}O_5$ (M_r 376.47) on the basis of single-crystal X-ray analyses, requiring seven degrees of unsaturation. The ^{1}H-NMR spectra of compound **1** showed signals of two secondary Me groups (δ(H) 1.08 and 1.09 (*d*, Me(18) and Me(19))), two tertiary Me groups (δ(H) 0.98 (*s*, Me(16)) and 1.55 (*s*, Me(20))), two olefinic H-atoms from an exo-CH_2 group (δ(H) 4.86 (*s*, H_a–C(15)) and 4.94 (*s*, H_b–C(15))), one O-

[1]) Arbitrary atom numbering. For systematic name and numbering, see *Exper. Part*.

bearing CH group (δ(H) 5.35 (*d*, *J* = 6.6, H–C(10))), one AcO group (δ(H) 2.10 (*s*)), and one terminal ^{i}Pr group (^{i}Pr–C(9)). The ^{13}C-NMR spectra of **1** revealed 22 C-atoms signals, corresponding to eight quaternary C-atoms, two CH groups, seven CH_2 groups, of one is olefinic, and five Me groups. The olefinic C-atoms from the exo-CH_2 group previously mentioned were detected at δ(C) 152.2 (C(1)) and 110.4 (C(15)), respectively. Further signals of a ketone CO group (δ(C) 207.1 (O=C(7))), two oxirane C-atoms (ethylene oxide) (δ(C) 73.2 (O–C(8)) and 79.4 (O–C(9))), one ester

Table 1. *^{1}H-NMR Data* ($CDCl_3$, 299.9 MHz) *and ^{13}C-APT* ($CDCl_3$, 75.0 MHz) *of Compound* **1**



1[1])

Position	δ(H)	^{1}H,^{1}H-COSY (H → H)	δ(C)	^{1}H,^{13}C-HSQC (H → C)	^{1}H, ^{13}C-HMBC (H → C)
1			152.2 (C)		2_α, 15a
2	2.52 (*ddd*, *J* = 13.5, 13.5, 6.9, H_α),	3, 2_β	31.9 (CH_2)	2_β, 2_α	15a, 15b
	2.10–2.12 (*m*, H_β)	2_α			
3	1.62–1.57 (*m*, 2 H)	2_α	22.6 (CH_2)	3	
4	1.95 (*ddd*, *J* = 12.6, 12.6, 6.0, 2 H)	3	37.8 (CH_2)	4	16, 6_β
5			41.8 (C)		16, 6_β, 6_α
6	3.74 (*d*, *J* = 15.3, 1 H, H_α),	6_β	54.5 (CH_2)	6_β, 6_α	16
	2.19 (*d*, *J* = 15.3, 1 H, H_β)	6_α			
7			207.1 (C)		
8			73.2 (C)		11_α, 20, 11_β, 6_β, 10
9			79.4 (C)		18, 19, 10
10	5.35 (*d*, *J* = 6.6, 1 H, H_α)	11_β	73.6 (CH)	10	
11	1.40 (*d*, *J* = 14.4, 1 H, H_α),	11_β	46.6 (CH_2)	11_α, 11_β	20
	1.71 (*d*, *J* = 7.2, 1 H, H_β)	10			
12			41.3 (C)		20, 13_β, 10
13	1.72 (*d*, *J* = 15.3, 1 H, H_α),	13_β	42.9 (CH_2)	13_α, 13_β	
	2.27 (*d*, *J* = 15.3, 1 H, H_β)	13_α			
14			77.1 (C)		16, 13_α, 6_β, 6_α, 15a, 15b
15	4.86 (*s*, 1 H, H_a),		110.4 (CH_2)	15a, 15b	2_α, 2_β
	4.94 (*s*, 1 H, H_b)				
16	0.98 (*s*, 3 H)		19.6 (Me)	16	6_α
17	2.73 (*sept.*, *J* = 6.9, 1 H)	18, 19	28.2 (CH)	17	18, 19
18	1.08 (*d*, *J* = 6.9, 3 H)	17	18.5 (Me)	18	19
19	1.09 (*d*, *J* = 6,9, 3 H)	17	20.4 (Me)	19	18
20	1.55 (*s*, 3 H)		24.9 (Me)	20	11_α, 11_β, 13_β
14-OH	not observed				
Me*C*O			169.9 (C)		*Me*CO, 10
*Me*CO	2.10 (*s*, 3 H)		21.6 (Me)	*Me*CO	

C=O group (δ(C) 169.9 (C(10)–O–*C*O–)), and one AcO group (δ(C) 21.6 (C(10)–O–CO–*Me*)) were revealed in the spectra (*Table 1*).

Comparison of these spectroscopic data with those of previously reported dolastane diterpenes [9] and analysis of the correlations observed in the HMBC and COSY spectra (*Fig. 2*) suggested a dolastane skeleton-type compound.

The COSY experiment of **1** showed two correlations between H–C(17)/Me(18) and H–C(17)/Me(19), suggesting the presence of a terminal ^{i}Pr fragment in the structure. No other COSY correlations were observed for H–C(17). Similarly, the CH_2 H-atom H_α–C(6) only showed one correlation with H_β–C(6), suggesting the absence of H-atoms in the close vicinity (*e.g.*, C(5), C(7), C(8), and C(9)), which was additionally confirmed by a ^{13}C-APT experiment that showed three O-bearing quaternary C-atom signals (δ(C) 41.8 (C(5)), 207.1 (C(7)), 73.2 (C(8)), and 79.4 (C(9))). Another important COSY correlation between a CH H-atom (δ(H) 5.35 (*d*, $J = 6.6$, H–C(10))) and a CH_2 H-atom (δ(H) 1.71 (*d*, H_β–C(11))) was observed. Additional correlations can be seen in *Table 1* and *Fig. 2*.

The HMBC spectrum of **1** indicated that sp^2 CH_2 H-atoms (H_a–C(15) and H_b–C(15)) displayed three correlations with C(1) (δ(C) 152.2, ^2J), C(2) (δ(C) 31.9, ^3J), and C(14) (δ(C) 77.1, ^3J). The tertiary Me(16) group showed four correlations with C(4) (δ(C) 37.8, ^3J), C(5) (δ(C) 41.8, ^2J), C(6) (δ(C) 54.5 ^2J), and C(14) (δ(C) 77.1, ^3J). Similarly, the tertiary Me(20) group displayed four correlations with C(11) (δ(C) 46.6, ^3J), C(12) (δ(C) 41.3, ^2J), C(13) (δ(C) 42.9, ^3J), and C(8) (δ(C) 73.2, ^3J). The quaternary C-atom C(12) exhibited correlations with a CH_2 H-atom (δ(H) 2.27 (H_α–C(13), ^2J)) and with a CH group (δ(H) 5.35 (H_β–C(10), ^3J)), which is correlated with the ester CO group at δ(C) 169.9 (^3J), which also displayed correlations with the AcO group signal (δ(C) 21.6 (^3J)). The epoxy C-atom C(8) (δ(C) 73.16) showed four correlations with Me(20) (^3J), H_α–C(11) and H_β–C(11) (^3J), and with an O-bearing CH group (δ(H) 5.35 (C(10), ^3J)). Similarly, the epoxy C-atom C(9) (δ(C) 79.4) also displayed correlations with C(10) (^2J), as well as with the the two secondary Me groups Me(18) and Me(19) (*Table 1*, *Fig. 2*).

The above-mentioned data allowed us to characterize compound **1** as 10β-acetoxy-8α,9α-epoxy-14β-hydroxy-7-oxodolastane, a new structure, which was confirmed by a single crystal X-ray structure analysis. The absolute configuration at C(5)[1]) (*R*), C(8) (*R*), C(9) (*S*), C(10) (*R*), C(12) (*S*), and C(14) (*S*)[1]), of compound **1** was also determined by full X-ray diffraction analysis (*Fig. 3*).

Due to the low yield of the purification process and the limited amount of starting material, only a few crystals of compound **1** were obtained with the slow evaporation of

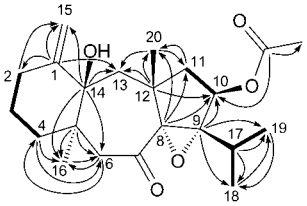


Fig. 2. *Selected COSY data* (—) *and HMBCs* (→) *observed for compound* **1**

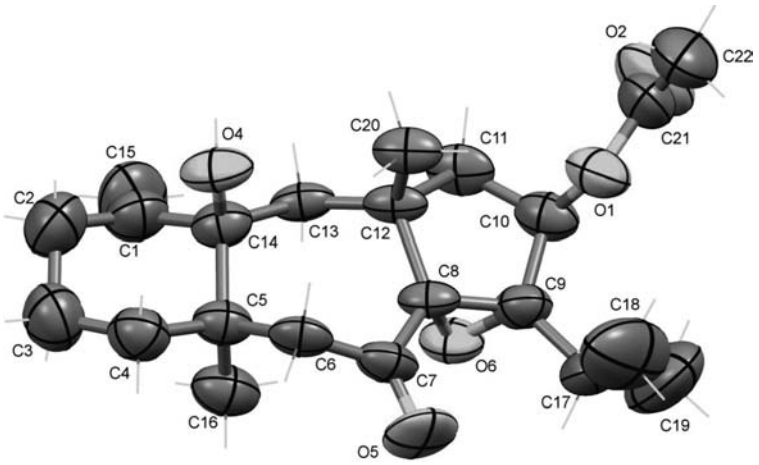


Fig. 3. *Three-dimensional structure of compound* **1***, obtained by single crystal X-ray diffraction analysis.* The atomic displacement ellipsoids are drawn at the 50% probability level.

CH_2Cl_2 at room temperature. Samples obtained in successive crystallization essays were investigated by single crystal X-ray diffraction techniques, but none of them diffracted with average $I/\sigma(I)$ ratio greater than 3 below 1.00 Å of resolution. Despite the low data quality, the structure elucidation and the structural refinement could be performed successfully. *Table 2* summarizes the crystal data and the structural refinement parameters.

The crystal packing of the structure of compound **1** is driven by weak hydrogen contacts and π-stacking contacts [22]. Details of the packing of **1** are depicted in *Fig. 4*. Intra- and intermolecular H-bonds are listed in *Table 3* and the atomic distances are listed in *Table 4*.

Many di- and tricyclic diterpenes have been isolated from the brown alga *C. cervicornis*. In a previous study, we reported the isolation and antifouling properties of the dolastane diterpenes (4*R*,9*S*,14*S*)-4α-acetoxy-9β,14α-dihydroxydolasta-1(15),7-diene, (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14α-hydroxydolasta-1(15),8-diene, and isolinearol, which inhibited the establishment of the mussel *Perna perna* [3]. In addition, we also showed that (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14α-hydroxydolasta-1(15),8-diene, the major compound from *C. cervicornis*, significantly inhibited feeding by the sea urchin *Lytechinus variegatus* [16]. Similarly, we assayed the same diterpene (4*R*,9*S*,14*S*)-4α-acetoxy-9β,14α-dihydroxydolasta-1(15),7-diene, which exhibited $IC_{50} = 2.2$, 12.0, and 4.0 μM for the promastigote, axenic amastigote, and intracellular amastigote forms of *Leishmania amazonensis*, respectively. In the following, we showed that the activity of this compound was 93 times less toxic to macrophages than that of protozoan and that the same compound induced a progressive loss of mitochondrial membrane potential and cell death in *L. amazonensis* [17]. In other studies, we showed the anticoagulant and antiplatelet activities [10], as well as the inhibition of mammal Na^+K^+-ATPase properties of (4*R*,9*S*,14*S*)-4α-acetoxy-9β,14α-dihydroxydolasta-1(15),7-diene, isolated from *C. cervicornis* [9]. Thus, we suggest that dolastane and *seco*-dolastane diterpene

Table 2. *Crystal Data and Structure Refinement for Compound* **1**

Empirical formula		$C_{22}H_{32}O_5$
Formula weight		376.47
Temperature [K]		293(2)
Wavelength [Å]		0.71073
Crystal system		Orthorhombic
Space group		$P2_12_12_1$
Unit cell dimensions:	a [Å]	6.1939(12)
	b [Å]	13.579(2)
	c [Å]	24.787(2)
V [Å^3]		2084.8(5)
Z		4
$D_{calc.}$ [g cm^{-3}]		1.199
Absorption coefficient [mm^{-1}]		0.084
$F(000)$		816
Crystal size [mm^3]		$0.05 \times 0.07 \times 0.15$
θ Range [°] for data collection		5.136 to 26.363
Index ranges		$-7 \le h \le 7,\ -16 \le k \le 15,\ -30 \le l \le 29$
Reflections collected		14883
Independent reflections		4176 ($R_{int} = 0.0783$)
Completeness to $\theta = 25.242°$		98.5%
Refinement method		Full-matrix least-squares on F^2
Data/restraints/parameters		4176/0/250
Goodness-of-fit on F^2		1.126
Final R indices [$I > 2\sigma(I)$]		R_1 [a]) = 0.0933, wR_2 [b]) = 0.2167
R Indices (all data)		$R_1 = 0.1552$, $wR_2 = 0.2433$
Absolute structure parameter		0.4(8)
Largest diff. peak and hole [e Å^{-3}]		0.310 and −0.296

[a]) $R_1 = \Sigma\,||F_0| - |F_c||\,/\Sigma\,|F_0|$. [b]) $wR_2 = \{\Sigma[w(F_0^2 - F_c^2)^2]/\Sigma[w(F_0^2)^2]\}^{1/2}$, where $w = 1/[\sigma^2(F_0^2) + (aP)^2 + bP]$ where a,b are adjustable constants and $P = (F_0^2 + 2\,F_c^2)/3$.

Table 3. *H-Bonds in Compound* **1**[1])

D–H···A[a])	D–H [Å]	H···A [Å]	D···A [Å]	Angle [°]
O(4)–H(4)···O(5)[b])	0.82	2.12	2.936(6)	175.3
C(17)–H(17)···O(5)[c])	0.98	2.46	3.008(11)	115.3
C(6)–H(6A)···O(6)[d])	0.97	2.66	3.510(8)	147.0
C(22)–H(22A)…O(2)[e])	0.96	2.69	3.562(11)	150.6

[a]) Atom code: D, donor; H, hydrogen; A, acceptor. [b]) Symmetry code: $-x + 2,\ y + 1/2,\ -z + 3/2$. [c]) Intramolecular H-bond. [d]) Symmetry code: $x + 1, y, z$. [e]) Symmetry code: $x + 1/2,\ -y + 3/2,\ -z + 1$.

compounds could play an important part of the defensive system of *C. cervicornis* against herbivores and foulant organisms, and therefore, there structures could be used as leading structures for new drugs. Consequently, new biological assays are needed to evaluate the biological activities of the new compound **1**.

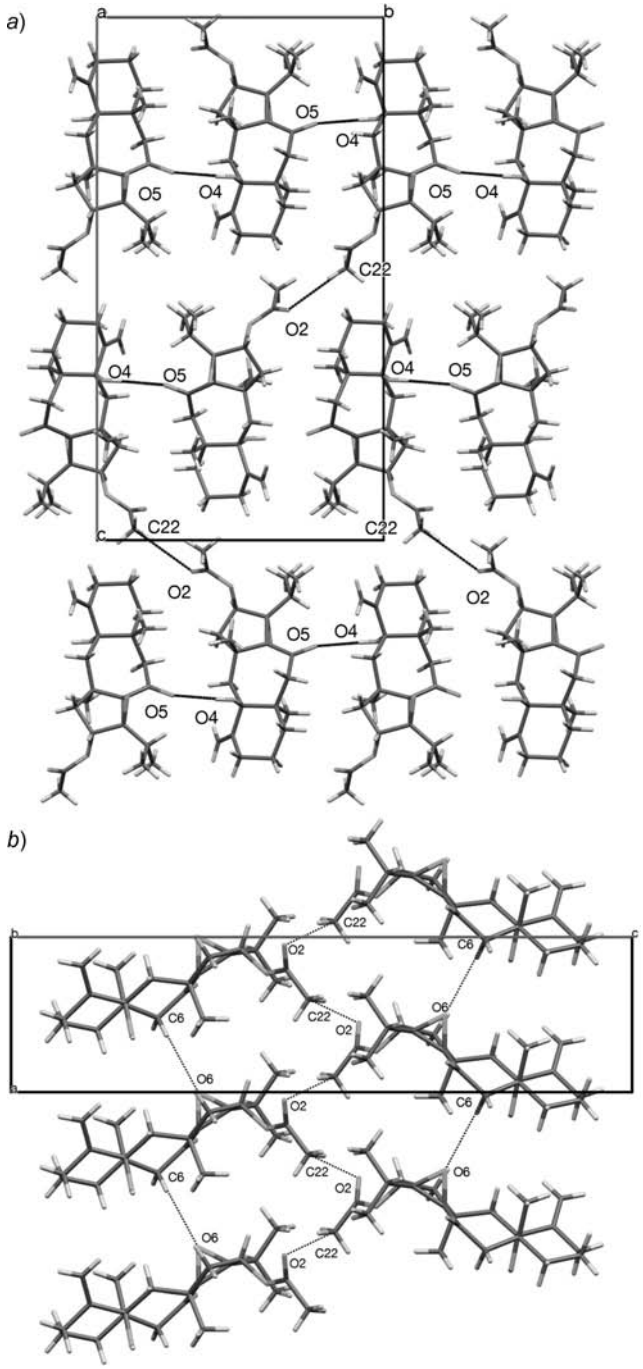


Fig. 4. *Three-dimensional crystallographic packing of compound* **1** *along* a *axis (*a*) and* b *axis (*b*)*. It is worth noting the O–H ··· O H-bonds between in-layer molecules and the weak Me ··· O and CH_2 ··· O H-bonds between molecules are from different layers.

Table 4. *C–C and C–O Interatomic Bond Lengths for Compound* **1**[1])

Bond	Distance [Å]	Bond	Distance [Å]
C1–C15	1.338(13)	C9–C17	1.508(10)
C1–C2	1.504(12)	C9–C10	1.533(9)
C1–C14	1.517(10)	C10–O1	1.459(9)
C2–C3	1.544(12)	C10–C11	1.518(10)
C3–C4	1.521(11)	C10–H10	0.9800
C5–C16	1.528(10)	C11–C12	1.542(9)
C5–C6	1.552(9)	C12–C13	1.532(10)
C5–C14	1.609(9)	C12–C20	1.554(9)
C6–C7	1.520(10)	C13–C14	1.525(9)
C7–O5	1.217(8)	C14–O4	1.428(8)
C7–C8	1.498(9)	C17–C18	1.499(16)
C8–O6	1.452(7)	C17–C19	1.551(16)
C8–C9	1.508(10)	C21–O2	1.181(11)
C8–C12	1.551(8)	C21–O1	1.372(10)
C9–O6	1.435(8)	C21–C22	1.489(14)

Conclusions. – In this study, a new dolastane diterpene 10β-acetoxy-8α,9α-epoxy-14β-hydroxy-7-oxodolastane (**1**) and four known *seco*-dolastane diterpenes (**2**–**5**) were isolated from the nonpolar extract of the Brazilian brown alga *Canistrocarpus cervicornis* (Dictyotaceae), collected in Rio de Janeiro's littoral. Their chemical structures were characterized by NMR spectroscopic techniques (1D and 2D), including a full X-ray diffraction analysis of compound **1**.

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Experimental Part

General. Materials and solvents were purchased from *Merck* (Germany), *Sinth* (Brazil), and *Vetec* (Brazil), and were used without further purification. Column chromatography (CC): silica gel (SiO_2) *Vetec* (70–230 mesh or 230–400 mesh). TLC (SiO_2; GF_{254}, *Merck*). ^{1}H-NMR (299.99 MHz) and ^{13}C-NMR (70.0 MHz): *Varian Unity Plus 300* spectromer using deuterated chloroform ($CDCl_3$; *Cambridge*) as solvent and TMS as internal standard; chemical shifts were reported in δ units [ppm] and coupling (J) in Hz. Diterpenes **2**–**5** were identified by comparison of physical and spectroscopic data with reported values [12][18][19].

X-Ray Crystallography. X-Ray diffraction data[2]) were performed on a *KAPPA* CCD diffractometer using graphite-Enhance Source MoK_α radiation ($\lambda = 0.7107$ Å). The measurement was performed at r.t. Data Integration and scaling of the reflections for all compounds were performed with the EvalCCD [23]. Final unit cell parameters were performed using Dirax/lsq [24] and no absorption correction was employed. Program XPREP [25] was used in the space group identification. The structure of **1** was solved by direct methods using the SIR92 [26] program. The positions of all atoms could be unambiguously

[2]) CCDC-1027234 (**1**) contains the supplementary crystallographic data for this article. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk./data_request/cif.

assigned on consecutive difference *Fourier* maps. Refinements were performed using SHELXL [25] based on F^2 through full-matrix least square routine. All non-H-atoms were refined with anisotropic atomic displacement parameters. The H-atoms in the compounds were added at idealized positions and further refined according to the riding model [27]. Distances of 0.97, 0.93, and 0.96 Å were restrained for C–H bonds of the CH_2, Me and aromatic C-atoms, resp., with $U_{iso}(H) = 1.2U_{eq}(C)$ for the CH_2 and aromatic C-atoms and $U_{iso}(H) = 1.5U_{eq}(O)$ for Me and OH groups. Molecular graphics were generated with Mercury [28].

Algal Collection. Specimens of the brown macroalga *C. cervicornis* (Dictyotaceae, Phaeophyta) were collected at Baía da Ribeira, Angra dos Reis City (lat. 23:00:24 S, long. 44:19:05 W), Rio de Janeiro State, Brazil, between the months of August and September, 2004, at depths between 1 and 4 m. The algal material was identified by Dr. *Joel Campos de Paula* at Universidade do Estado do Rio de Janeiro, Brazil, and voucher specimens (HRJ10772) were deposited with the Herbarium of the Universidade do Estado do Rio de Janeiro, Brazil.

Extraction and Isolation. The air-dried and powdered algal material (900 g) was extracted with CH_2Cl_2 at r.t. during 28 d. Subsequently, the solvent was removed under reduced pressure yielding 44 g of CH_2Cl_2 crude extract (DCE). This residue was subjected to FC (SiO_2; 7 × 10 cm) eluted with hexane (*A*), $CHCl_3$ (*B*), AcOEt (*C*), acteone (*D*), and MeOH (*E*), yielding five fractions of 500 ml each (F_A–F_E). F_B and F_C were analyzed by TLC and ^{1}H-NMR analyses and indicated the presence of *seco*-dolastane diterpenes. Thus, a part of F_C (5 g) was subjected to prep. CC (SiO_2; 4 × 28 cm) with $CHCl_3$/AcOEt 95:5 with an increase in polarity 5% until 100% AcOEt, to give 143 fractions (F_{C1}–F_{C143}, 20 ml each). The fractions F_{C98}–F_{C117} were combined to produce a brownish residue (0.5 g) and it was subjected to prep. CC (SiO_2; 3 × 15 cm) with CH_2Cl_2/AcOEt 7:3, to give 75 fractions ($F_{C1'}$–$F_{C75'}$). The fractions $F_{C26'}$ and $F_{C27'}$ afforded the pure compound **2** (25 mg, yellow gum). A part of F_B (250 mg) was submitted to prep. CC (SiO_2; 1.5 × 20 cm) with hexane/CH_2Cl_2 1:1, CH_2Cl_2, acteone, and acetone/MeOH 1:1 to give 26 fractions (F_{B1}–F_{B26}, 7 ml each). The fractions F_{B24} and F_{B25} were combined and afforded the *seco*-dolastane **3** (27 mg, yellow gum). Another part of F_B (500 mg) was subjected to prep. CC (SiO_2; 4 × 28 cm) eluted with $CHCl_3$/AcOEt 95:5 with increasing of polarity 5% until 100% AcOEt to give 72 fractions ($F_{B1'}$–$F_{B72'}$, 20 ml each). All fractions collected were analyzed by TLC and $F_{B28'}$–$F_{B62'}$ were combined to produce a yellow residue (270 mg). This mixture was subjected to prep. CC (SiO_2; 4 × 15 cm) with hexane/AcOEt 7:3 to give 30 fractions ($F_{B1''}$–$F_{B30''}$, 20 ml each). From these proceedings, the fractions $F_{B14''}$ and $F_{B15''}$ afforded compound **4** (18 mg, yellow gum), and the similar fractions $F_{B16''}$ and $F_{B17''}$ (65 mg) were combined after analysis by TLC, to give a mixture which was submitted to prep. CC (SiO_2; 1 × 21 cm) with hexane/AcOEt 7:3, to give 18 fractions ($F_{B1'''}$–$F_{B18'''}$, 5 ml each). From this fractioning, the fraction $F_{B2'''}$ afforded the pure compound **5** (8.4 mg, colorless oil). To isolate compound **1**, a part of F_B (430 mg) was submitted to prep. CC (SiO_2; 2 × 34 cm) with hexane/AcOEt 9:1, to give 70 fractions (F_{B1*}–F_{B70*}, 20 ml each). The fractions F_{B15*}–F_{B32*} were combined (295 mg) and submitted two times to CC (SiO_2; 2 × 34 cm) with hexane/AcOEt 5:1, 4:1 and 3:1. Fractions eluted with hexane/AcOEt (5:1) afforded the pure compound **1** (1.5 mg, colorless crystal).

10β-Acetoxy-8α,9α-epoxy-14β-hydroxy-7-oxodolastane (=*(2*R*,3a*S*,4a*R*,8a*R*,10a*R*)-Dodecahydro-4a-hydroxy-3a,8a-dimethyl-5-methylidene-10-oxo-1a-(propan-2-yl)-1a*H*-benzo[5,6]azuleno[1,8a-*b*]oxiren-2-yl Acetate*; **1**). M.p., IR, UV, and EI-MS analysis could not be determined due to scarcity of material. ^{1}H- and ^{13}C-NMR: see *Table 1*. X-Ray data: *Tables 2–4*.

Isolinearol (**2**). ^{1}H-NMR ($CDCl_3$): 4.97 (*s*, H_b–C(15)); 4.82 (*s*, H_a–C(15)); 3.58 (*t*, *J* = 3.0, H–C(4)); 2.74 (*ddd*, *J* = 13.5, 13.5, 7.2, H_α–C(2)); 2.62 (*sept.*, *J* = 6.9, H–C(17)); 2.52 (*dd*, *J* = 9.6, 6.3, CH_2(7)); 2.26 (*dd*, *J* = 12.6, 6.9, H_β–C(11)); 2.14 (*dd*, *J* = 13.5, 5.4, H_β–C(2)); 1.99 (*d*, *J* = 13.5, H_β–C(13)); 1.92 (*dd*, *J* = 14.4, 6.9, H_α–C(11)); 1.86–1.84 (*m*, CH_2(3)); 1.80–1.76 (*m*, CH_2(10)); 1.76 (*dd*, *J* = 6.3, 3.3, CH_2(6)); 1.70 (*d*, *J* = 13.5, H_α–C(13)); 1.12 (*d*, *J* = 6.9, Me(18)); 1.11 (*d*, *J* = 6.9, Me(19)); 1.02 (*s*, Me(20)); 0.67 (*s*, Me(16)). ^{13}C-NMR ($CDCl_3$): 214.8 (C(9)); 146.6 (C(1)); 110.0 (C(15)); 105.4 (C(8)); 85.0 (C(14)); 78.5 (C(4)); 43.2 (C(12)); 40.9 (C(17)); 40.8 (C(13)); 39.6 (C(5)); 35.7 (C(7)); 33.0 (C(3)); 31.7 (C(10)); 31.0 (C(11)); 29.5 (C(6)); 27.2 (C(2)); 22.4 (C(16)); 18.19 (C(18)); 18.23 (C(19); 22.3 (C(20)).

Isolinearol Acetate (**3**). ^{1}H-NMR ($CDCl_3$): 4.96 (br. *s*, H_b–C(15)); 4.85 (br. *t*, H–C(4)); 4.82 (br. *s*, H_a–C(15)); 2.63 (*sept.*, *J* = 6.9, H–C(17)); 2.18 (*s*, MeCO); 1.11 (*d*, *J* = 6.9, Me(18), Me(19)); 1.02 (*s*, Me(20)); 0.78 (*s*, Me(16)).

Linearol (**4**). ^{1}H-NMR ($CDCl_3$): 5.13 (*s*, H_b–C(15)); 4.89 (*s*, H_a–C(15)); 4.23 (*t*, *J* = 2.7, H–C(2)); 3.75 (br. *s*, HO–C(2)); 2.60 (*sept.*, *J* = 6.9, H–C(17)); 1.84 – 1.80 (*m*, H–C(3)); 1.12 (*d*, *J* = 6.9, Me(19)); 1.11 (*d*, *J* = 6.9, Me(18)); 1.02 (*s*, Me(20)); 0.71 (*s*, Me(16)).

Indicol (**5**). ^{1}H-NMR ($CDCl_3$): 4.86 (br. *s*, H_b–C(15)); 4.69 (br. *s*, H_a–C(15)); 2.62 (*sept.*, *J* = 6.9, H–C(17)); 1.12 (*d*, *J* = 6.9, Me(19)); 1.11 (*d*, *J* = 6.9, Me(18)); 1.01 (*s*, Me(20)); 0.72 (*s*, Me(16)). ^{13}C-NMR ($CDCl_3$): 214.8 (C(9)); 148.4 (C(1)); 108.3 (C(15)); 104.7 (C(8)); 84.0 (C(14)); 44.0 (C(12)); 40.9 (C(17)); 40.6 (C(13)); 36.9 (C(5)); 36.1 (C(3)); 34.3 (C(7)); 32.5 (C(10), C(11)); 29.8 (C(6)); 28.6 (C(2)); 23.2 (C(4)); 22.8 (C(20)); 21.4 (C(16)); 18.3 (C(19)); 18.2 (C(18)).

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