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Aldingenin derivatives from the red alga *Laurencia aldingensis*

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Abstract

Three brominated bisabolene-type sesquiterpene derivatives, aldingenin B, C and D, together with cholesterol and palmitic acid, have been isolated from the red alga *Laurencia aldingensis* (Ceramiales, Rodophyta) and their structures elucidated by spectroscopic methods including NMR analysis.

Keywords: *Laurencia aldingensis*; Sesquiterpenes; Brominated derivatives

1. Introduction

Red algae of the *Laurencia* Lamouroux complex are well-known as prolific sources of halogenated secondary metabolites, particularly of terpenoids and C_{15} acetogenins. More than 500 different secondary metabolites have been characterised in members of this genus of marine algae (Fenical and Norris, 1975; Martın and Darias, 1978; Erickson, 1983), and most species elaborate at least one compound that is not found in any of the others (Faulkner, 1996; Masuda et al., 1996, 1997). Thus, halogenated secondary metabolites are considered to be useful taxonomic markers for the discrimination of species within the *Laurencia* complex.

Previously, we have isolated the novel sesquiterpene aldingenin A from Brazilian specimens of *Laurencia aldingensis* Saito & Womersley (Carvalho et al., 2003), a species originally described from the Aldinga Isles, Australia (Saito and Womersley, 1974). In this paper, we report the isolation and structural determination of a further three new sesquiterpenes, aldingenin B (**1**), aldingenin C (**2**) and aldingenin D (**3**), together with cholesterol and palmitc acid, from the same algal source. Compounds **1**–**3** belong to a novel oxacyclic class of bisabolane-type derivatives, the distribution of which appears to be restricted to *L. aldingensis*.

2. Results and discussion

The CH_2Cl_2 extract of *L. aldingensis* was submitted to chromatographic separation over silica gel and Sephadex LH-20 to afford aldingenin B (**1**), aldingenin C (**2**) and aldingenin D (**3**) (Fig. 1), each of which was obtained in the form of a white amorphous solid.

The HREIMS of **1** presented a molecular ion at *m*/*z* 346.0748 corresponding to the molecular formula $C_{15}H_{23}O_4Br$ with four degrees of unsaturation. In the IR spectrum, the absence of signals associated with double bonds or carbonyl groups indicated that **1** was tetracyclic, whilst a broad band at 3450 cm^{-1} implied the presence of an hydroxyl group. The ^{13}C NMR spectra (BBD, DEPT 135° and DEPT 90°) displayed 15 signals (Table 1) corresponding to four methyl, three methylene and four methine

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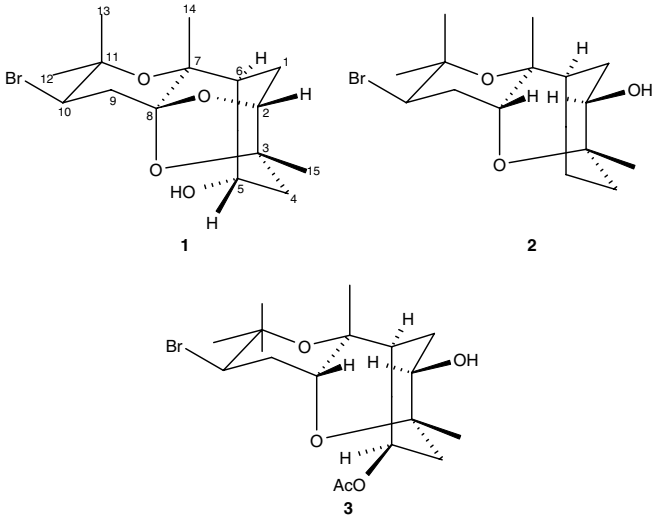

Fig. 1. Novel sesquiterpenes aldingenin B (**1**), aldingenin C (**2**) and aldingenin D (**3**) isolated from *Laurencia aldingensis*.

Table 1
^{13}C NMR spectroscopic data (125 MHz) for aldingenin B (**1**), C (**2**) and D (**3**)

No.	**1**[a]	**2**[b]	**3**[b]
1	31.3 (CH_2)	36.4 (CH_2)	32.5 (CH_2)
2	60.3 (CH)	63.5 (CH)	62.7 (CH)
3	69.3 (C)	71.8 (C)	70.7 (C)
4	44.3 (CH_2)	42.9 (CH_2)	46.5 (CH_2)
5	68.9 (CH)	22.7 (CH_2)	69.3 (CH)
6	47.8 (CH)	45.9 (CH)	46.4 (CH)
7	78.5 (C)	77.2 (C)	76.6 (C)
8	107.0 (C)	70.9 (CH)	70.6 (CH)
9	39.5 (CH_2)	36.3 (CH_2)	35.9 (CH_2)
10	52.7 (CH)	52.9 (CH)	52.3 (CH)
11	76.2 (C)	75.6 (C)	75.7 (C)
12	29.6 (CH_3)	31.1 (CH_3)	30.9 (CH_3)
13	23.2 (CH_3)	24.1 (CH_3)	24.0 (CH_3)
14	22.2 (CH_3)	24.2 (CH_3)	21.5 (CH_3)
15	30.7 (CH_3)	19.9 (CH_3)	27.5 (CH_3)
C(O)	–	–	169.3 (C)
CH_3	–	–	21.4 (CH_3)

[a] Compound dissolved in benzene-d_6.
[b] Compound dissolved in $CDCl_3$.

carbons (three of these bearing heteroatoms) and four non-protonated carbons (all bearing heteroatoms). The ^{1}H NMR spectrum (C_6D_6) of **1** (Table 2) exhibited four methyl singlets at δ 1.19, 1.36, 1.49 and 1.57, whilst the resonances at δ 3.99 (1H, *dd*, $J = 9.6$ and 6.3 Hz) and δ 3.86 (1H, *ddd*, $J = 9.6$, 8.4 and 4.7 Hz) could be assigned to protons on oxygenated carbons. The signal at δ 4.17 (1H, *dd*, $J = 13.5$ and 3.5 Hz) was attributed to the proton on the brominated carbon as in aldingenin A (Carvalho et al., 2003). All of the resonances associated with the hydrogen-bearing carbons in **1** were assigned from the HMQC spectrum.

The basic skeleton of **1** was established from analysis of the NMR spectroscopic data (COSY, HMQC and HMBC) following the procedures previously employed in the structural elucidation of aldingenin A (Carvalho et al., 2003).

Table 2
^{1}H NMR spectroscopic data (500 MHz) for aldingenin B (**1**), C (**2**) and D (**3**) in $CDCl_3$

No.	**1**[a]	**2**[b]	**3**[b]
1	1.65 *m*	2.28 *m*	2.30 *dt* (11.7, 2.8)
		1.66 *m*	1.42 *m*
2	3.99 *dd* (9.6, 6.3)	4.37 *dd* (12.4, 4.5)	4.44 *dd* (11.7, 4.9)
4	1.92 *dd* (14.5, 9.6)	2.40 *dt* (13.4, 3.3)	2.74 *dd* (15.0, 3.1)
	2.16 *dd* (14.5, 4.7)	2.03 *td* (13.4, 3.3)	2.20 *m*
5	3.86 *ddd* (9.6, 8.4, 4.7)	1.88 *m*	5.44 *d* (2.6)
		1.22 *m*	
6	1.44 *dd* (9.0, 8.4)	1.86 *m*	2.34 *m*
8	–	3.57 *dd* (4.0, 2.5)	3.58 *t* (3.3)
9	2.19 *t* (13.5)	2.51 *td* (13.7, 2.5)	2.56 *td* (13.3, 2.4)
	1.72 *dd* (13.5, 3.5)	2.22 *dt* (13.7, 4.0)	2.19 *m*
10	4.17 *dd* (13.5, 3.5)	4.29 *dd* (13.7, 4.0)	4.27 *dd* (13.3, 4.1)
12	1.36 *s*	1.32 *s*	1.31 *s*
13	1.49 *s*	1.38 *s*	1.34 *s*
14	1.19 *s*	1.14 *s*	1.18 *s*
15	1.57 *s*	1.69 *s*	1.78 *s*
CH_3	–	–	2.05 *s*

[a] Compound dissolved in benzene-d_6.
[b] Compound dissolved in $CDCl_3$.

The ^{1}H–^{1}H COSY spectrum of **1** indicated three proton sequences. The connectivities between signals at δ 2.19, 1.72 and 4.17 showed that H-9a/H-9b and H-10 were adjacent to each other and that C-8 was a non-protonated carbon. Correlation of the signal at δ 3.86 and those at δ1.44, 1.92 and 2.16 indicated, respectively, the connectivities of H-5/H6, H-5/H-4a and H-5/H-4b. Finally, the proton sequence between the signals at δ 3.99 and 1.65 revealed the connectivity of H-2/H-1. These correlations confirmed the presence in **1** of the sesquiterpene skeleton previously established for aldingenin A. Additional assignments derived from the observed correlations in the HMBC spectrum (Fig. 2) between the signals at δ 4.17 (H-10), δ 1.19 (H-14) and δ 1.44 (H-6) and that at δ 107.0 (C-8). As the chemical shift assigned to C-8 was characteristic of an acetal carbon, a further ether ring could be established between C-8 and the oxymethine carbon C-2 to form a tetracyclic compound. The hydroxyl group indicated in the IR spectrum of **1** should, therefore, be located at C-5 on the remaining oxymethine carbon.

The relative stereochemistry of **1** was assigned on the basis of correlations observed in the NOESY spectrum (Fig. 3) and on an analysis of the coupling constants. As previously described for aldingenin A (Carvalho et al., 2003), the coupling constant of H-10 is typical of an axial hydrogen, indicating an equatorial position for the bromine atom. In the NOESY spectrum, strong correlations between H-10/H-12, H-10/H-9a, H-13/H-9b and H-13/H-14 were observed signifying spatial arrangements of the C-12, C-13 and C-14 methyl groups and of the bromine atom equivalent to those established for aldingenin A. Additionally, the cross-peaks between H-15/H-2, H-15/H-4a and H-4b/H-5 were indicative of an S* configuration at C-5. All of these data confirmed the structure of compound **1**, named aldingenin B, as presented in Fig. 1.

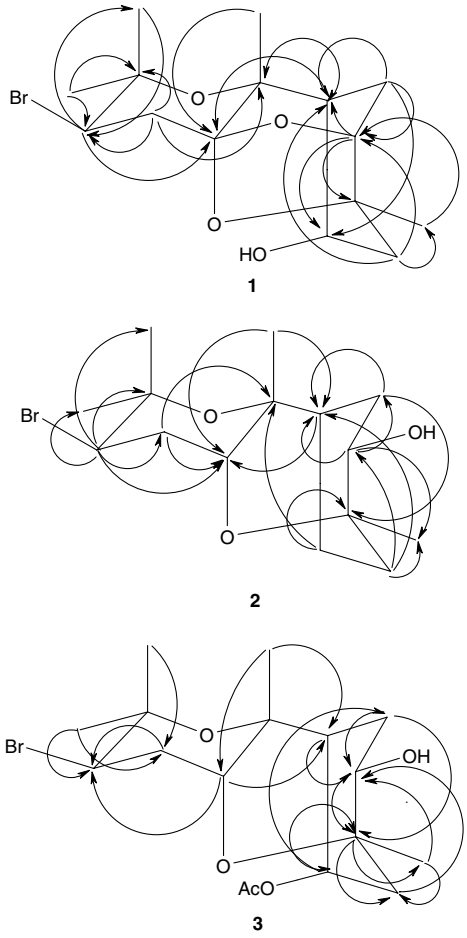

Fig. 2. Major HMBC correlations (H → C) observed in the sesquiterpenes **1**, **2** and **3**.

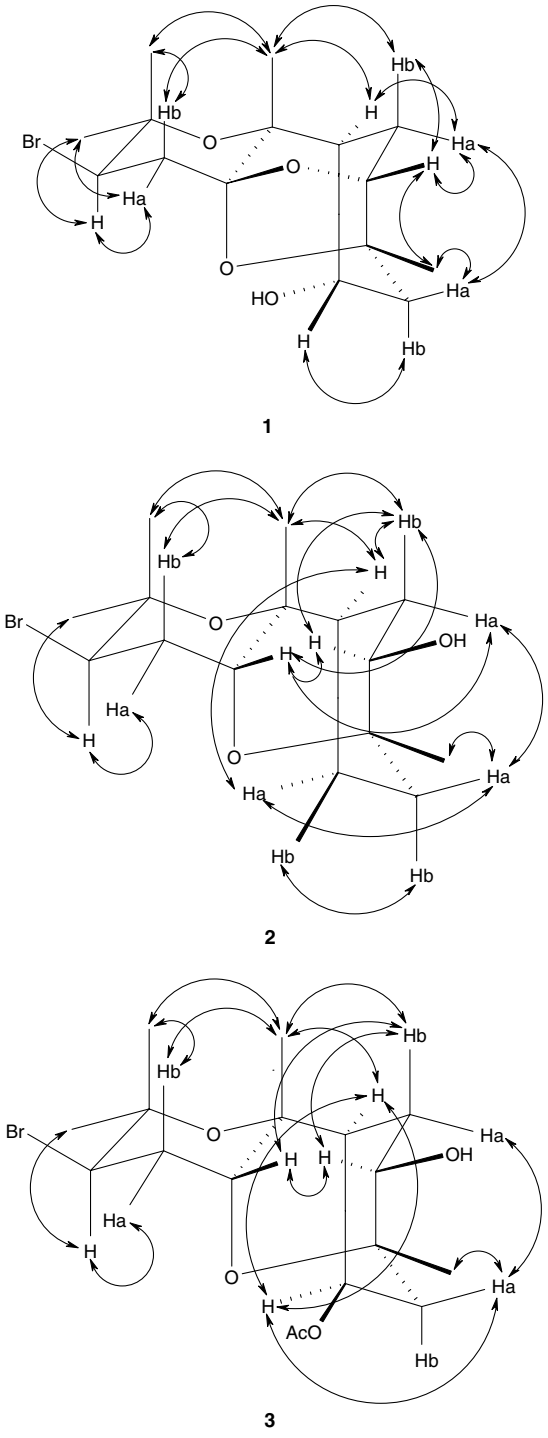

Fig. 3. Major NOE correlations observed in the NOESY spectra of **1**, **2** and **3**.

Compound **2** displayed a molecular ion at m/z 332.0971 in the HREIMS corresponding to the molecular formula $C_{15}H_{25}O_3Br$ with three degrees of unsaturation. The IR spectrum exhibited a strong absorption at 3455 cm^{-1} consistent with the presence of an hydroxyl group. The ^{13}C NMR spectra (BBD, DEPT 135° and DEPT 90°) revealed the presence of four methyl carbons with signals at δ 19.9, 24.1, 24.2 and 31.1, four methylene carbons at δ 22.7, 36.3, 36.4 and 42.9, one methine carbon at δ 45.9, one methine carbon bearing a bromine atom at δ 52.9, and one oxymethine carbon at δ 63.5. Three non-protonated carbons, each bearing an oxygen atom, afforded signals at δ 71.8, 75.6 and 77.2. All of the resonances associated with the hydrogen-bearing carbons in **2** were assigned from the HMQC spectrum.

A comparison of the NMR spectroscopic data of **2** with those of **1** and aldingenin A (Carvalho et al., 2003) suggested the same structural skeleton for these molecules. Thus, the ^1H NMR spectrum of **2** ($CDCl_3$) showed a characteristic downfield signal at δ 4.29 (*dd*, $J = 13.7$ and 4.0 Hz) that was assigned to the proton of the carbon bearing the bromine atom (H-10). The correlation between resonances at δ 4.29 (H-10) and δ 2.51/2.22 (H-9), and between signals at δ 2.51/2.22 (H-9) and δ 3.57 (H-8) observed in the ^1H–^1H COSY spectrum indicated a fragment (moiety **a**) similar to that observed in aldingenin A (Carvalho et al., 2003). In the ^1H–^1H COSY spectrum, there were also cross-peaks between the signals at δ 4.37 (H-2) and δ 2.28 (H-1a)/1.66 (H-1b), and between the latter and δ 1.86 (H-6). These, together with the correlations observed between the resonance at δ 1.86 (H-6) and those at δ 1.88 (H-5a)/1.22 (H-5b), and between these signals

and δ 2.40 (H-4a)/2.03 (H-4b), indicated a proton sequence H-2/H-1/H-6/H-5/H-4. Finally, the location of the hydroxyl group at C-2 was established from an HMBC experiment in which a correlation between the resonance at δ 4.37 (H-2) and the signals at δ 19.9 (C-15) and δ 45.9 (C-6) were observed. Full HMBC analysis, therefore, confirmed the structure of compound **2**, named aldingenin C, as presented in Fig. 2.

The relative stereochemistry of **2** was assigned on the basis of an analysis of the coupling constants and of the correlations observed in the NOESY spectrum (Fig. 3). The chemical shift and coupling constants of H-10 were similar to those observed in other aldingenin derivatives indicating an equatorial position for the bromine atom. In the NOESY spectrum, strong correlations between H-10/H-12, H-10/H-9a, H-13/H-9b and H-13/H-14 were also observed signifying spatial arrangements of the C-12, C-13 and C-14 methyl groups and of the bromine atom equivalent to those established for **1** and aldingenin A. Additionally, the cross-peaks between H-8/H-2, H-8/H-1b and between H-1b/H-2 and H-1b/H-6 were indicative of a 2R* configuration for **2**.

The HREIMS of **3** exhibited a molecular ion at *m/z* 390.1059 corresponding to the molecular formula $C_{17}H_{27}O_5Br$ with four degrees of unsaturation. The IR spectrum revealed absorption bands for hydroxyl (3465 cm^{-1}) and carbonyl (1735 cm^{-1}) groups. The ^{13}C NMR spectra (BBD, DEPT 135° and DEPT 90°) exhibited a signal at δ 169.3 (C) which, in association with the signal at δ 21.4 (CH_3), was assigned to an acetoxyl group. These spectra also showed four methyl groups with signals at δ 21.5, 24.0, 27.5 and 30.9, six carbons (each bearing an oxygen atom) at δ 62.7 (CH), 69.3 (CH), 70.6 (CH), 70.7 (C), 75.7 (C), 76.6 (C) and one carbon bearing a bromine atom at δ 52.3 (CH). Together with these resonances, those of three methylene carbons at δ 32.5, 35.9 and 46.5, and one methine carbon at δ 46.4, were also observed. Comparison of the NMR spectroscopic data of **1**, **2** and aldingenin A (Carvalho et al., 2003) with those of **3** indicated that all possessed the same structural skeleton. The ^{1}H–^{1}H COSY spectrum showed cross-peaks between the proton at δ 4.27 (H-10) and that at δ 2.56/2.19 (H-9), whilst the latter was also correlated with signals at δ 3.58 (H-8) and δ 4.27 (H-10). These findings indicated the proton sequence H-10/H-9/H-8, establishing the presence of moiety **a**, similar to that defined previously for aldingenin A (Carvalho et al., 2003). The HMBC spectrum (^3J) showed a correlation between the signal of the tertiary carbinolic carbon at δ 70.7 (C-3) with resonances at δ 2.30/1.42 (H-1) and 5.44 (H-5), whilst the spectrum recorded at ^2J showed that the signal at δ 70.7 (C-3) was associated with protons at δ 1.78 (H-15), 2.74/2.20 (H-4) and 4.44 (H-2). These correlations, together with the analysis of the HMQC spectrum, allowed the assignment of C-2 at δ 62.7 and C-5 at δ 69.3. The chemical shift for carbon C-5 (δ_C 69.3), and the respective proton H-5 (δ_H 5.44), indicated that the hydroxyl group on this carbon was acetylated. The complete HMQC and HMBC analysis confirmed the structure of compound **3**, which has been named aldingenin D.

Analysis of the NOESY spectrum of **3** (Fig. 3) showed cross-peaks between H-10/H-12, H-10/H-9a, H-13/H-9b, H-13/H-14 and H-14/H-6 that were indicative of the same spatial arrangements of the C-7 and C-11 methyl groups and of the bromine atom at C-10 as have been established for other aldingenin derivatives. The configuration of the hydroxyl group at C-2 was defined as R* based on correlations between H-8/H-2, H-8/H-1b and between H-1b/H-2 and H-1b/H-6, which were similar to those observed for **1**. The cross-peaks between H-5/H-6, H-5/H-4b and between H-4a/H-15 indicated an R* configuration at C-5.

The known compounds cholesterol and palmitic acid were identified by comparison of their ^{1}H NMR spectra and LREIMS data with those reported (Granato et al., 2000; Chaves et al., 2004) and by direct comparison with standard compounds.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively, on a Bruker model DRX-500 spectrometer with samples dissolved in $CDCl_3$ or benzene-d_6 (Aldrich). TMS was employed as internal standard: chemical shifts were recorded in δ (ppm) and coupling constants (J) in Hz. IR spectra (film) were determined on a Perkin–Elmer model 1750 spectrometer. LREIMS and HREIMS spectra were measured at 70 eV on Finnigan-Mat INCOS 50 quadrupole and VG Autospec spectrometers, respectively. Optical rotations were determined using a Jasco DIP-370 digital polarimeter (Na filter, $\lambda = 588$ nm). Silica gel (Merck, 230-400 mesh) and Sephadex LH-20 (Sigma) were employed in the CC separations, whilst analytical TLC was performed using silica gel 60 PF_{254} layers (Merck).

3.2. Algal material

Specimens of *L. aldingensis* for morphological and chemical studies were collected at Castelhanos (Espirito Santo, Brazil) in June 1995 and in June 1997. Voucher specimens (SP-365655 and SP-355409, respectively) were deposited in the Herbarium Maria Eneida P. Kauffman Figalgo of the Instituto de Botânica (SMA), São Paulo, Brazil.

3.3. Extraction and isolation

Material for chemical analysis was washed with distilled H_2O, air-dried at room temperature (20–25 °C during 48 h), and a sample (153 g) extracted with CH_2Cl_2 (4 × 500 mL). After filtration, the combined extracts were evaporated to yield a yellow oily material (1.28 g) that

was separated by silica gel CC with a step gradient of hexane and EtOAc to give three main fractions 1–3. The first fraction (72 mg) was submitted to CC over silica gel with CH_2Cl_2:hexane (4:1, v/v) as eluent yielding three sub-fractions *I–III*. Sub-fractions *I* and *II* contained palmitic acid (10.0 mg) and cholesterol (8.1 mg), respectively. Sub-fraction *III* comprised crude aldingenin B (**1**) and was purified by CC over Sephadex LH-20 with CH_2Cl_2:MeOH (1:1, v/v) as eluent to give pure **1** (2.0 mg). Repeated CC of fraction *2* over silica gel with a gradient of CH_2Cl_2 and MeOH and with $CHCl_3$:*iso*-propanol (99:1, v/v), yielded aldingenin D (**3**) (2.4 mg). Pure aldingenin C (**2**) (1.5 mg) was obtained from fraction *3* after purification over silica gel column with gradient of CH_2Cl_2 and MeOH.

3.4. Aldingenin B (*1*)

White amorphous solid. $[\alpha]_D^{25}$ −105.9 (*c* 0.08, MeOH). IR ν^{film} cm^{-1} 3450, 2984, 2944, 2860, 1365, 1129, 1060, 932, 742. For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2. HREIMS *m/z* 346.0748 (calcd. for $C_{15}H_{23}O_4{}^{79}Br$, 346.0780).

3.5. Aldingenin C (*2*)

White amorphous solid. $[\alpha]_D^{25}$ −54.5 (*c* 0.06, MeOH). IR ν^{film} cm^{-1} 3455, 2982, 2947, 2867, 1460, 1382, 1124, 1069, 971, 733. For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2. HREIMS *m/z* 332.0971 (calcd. for $C_{15}H_{25}O_3{}^{79}Br$, 332.0987).

3.6. Aldingenin D (*3*)

White amorphous solid. $[\alpha]_D^{25}$ +48.0 (*c* 0.12, MeOH). IR ν^{film} cm^{-1} 3465, 2925, 2857, 1735, 1459, 1380, 1244, 1071, 749. For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1 and 2. HREIMS *m/z* 390.1059 (calcd. for $C_{17}H_{27}O_5{}^{79}Br$, 390.1042).

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References

Carvalho, L.R., Fujii, M.T., Kato, M.J., Roque, N.F., Lago, J.H.G., 2003. Aldingenin A, new brominated sesquiterpene from red algae *Laurencia aldingensis*. Tetrahedron Letters 44, 2637–2640.

Chaves, M.H., Barbosa, A.S., Moita Neto, J.M., Aued-Pimentel, S., Lago, J.H.G., 2004. Caracterização química do óleo da amêndoa de *Sterculia striata* St. Hi. et Naud. Química Nova 27, 404–408.

Erickson, K.L., 1983. In: Scheuer, P.J. (Ed.), Marine Natural Products, vol. 5. Academic Press, New York.

Faulkner, D.J., 1996. Marine natural-products. Natural Product Reports 13, 75–125.

Fenical, W., Norris, J.N., 1975. Chemotaxonomy in marine algae: chemical separation of some *Laurencia* species (Rhodophyta) from the Gulf of California. Journal of Phycology 11, 104–108.

Granato, A.C., Berlinck, R.G.S., Magalhães, A., Schefer, A.B., Ferreira, A.G., Sanctis, B., Freitas, J.C., Hajdu, E., Migotto, A.E., 2000. Produtos naturais das esponjas marinhas *Aaptos* sp., *Hymeniacidon* aff. *heliophila*, e do nudibrânquio *Doris* aff *verrucosa*. Química Nova 23, 594–599.

Martín, J.D., Darias, J., 1978. In: Scheuer, P.J. (Ed.), Marine Natural Products, vol. 1. Academic Press, New York.

Masuda, M., Abe, T., Suzuki, T., Suzuki, M., 1996. Morphological and chemotaxonomic studies on *Laurencia composita* and *L. okamurae* (Ceramiales, Rhodophyta). Phycologia 35, 550–562.

Masuda, M., Abe, T., Sato, S., Suzuki, T., Suzuki, M., 1997. Diversity of halogenated secondary metabolites in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales). Journal of Phycology 33, 196–208.

Saito, Y., Womersley, H.B.S., 1974. The southern Australian species of *Laurencia* (Ceramiales: Rhodophyta). Australian Journal of Botany 22, 815–874.