Cytotoxic Halogenated Metabolites from the Brazilian Red Alga *Laurencia catarinensis*

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Seven new (**1**−**7**) and seven previously reported (**8**−**14**) halogenated metabolites were isolated from the organic extract of the Brazilian red alga *Laurencia catarinensis*. The structure elucidation and the assignment of the relative configurations of the new natural products were based on detailed NMR and MS spectroscopic analyses, whereas the structure of metabolite **6** was confirmed by single-crystal X-ray diffraction analysis. The absolute configuration of metabolite **1** was determined using the modified Mosher's method. The in vitro cytotoxicity of compounds **1**−**14** was evaluated against HT29, MCF7, and A431 cell lines.

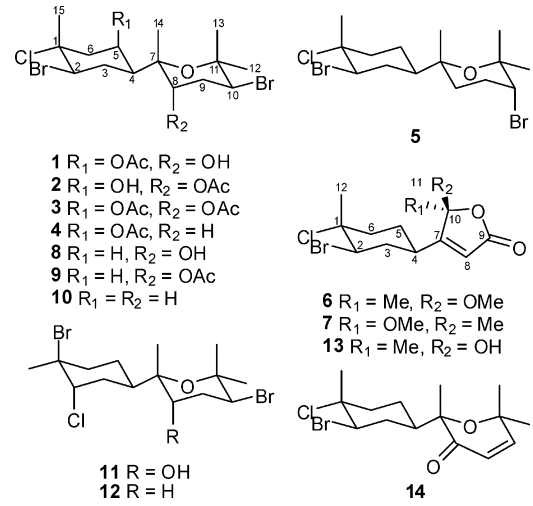
Red algae of the genus *Laurencia* (Rhodomelaceae) are cosmopolitan species with a wide distribution throughout the world. Even though they have been the subject of intense research over the last fifty years, they are still a prolific source of new natural products in the marine environment. Their secondary metabolites include sesquiterpenes, diterpenes, triterpenes, and acetogenins that are usually characterized by the presence of one or more halogen atoms in their structures.[1,2] Due to their relatively high degree of halogenation, many of these molecules either are biologically active or play an ecological role in their ecosystem, often exhibiting antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, cytotoxic, antifouling, antifeedant, ichthyotoxic, and/or insecticidal activity.[1,2] At the same time, insight on the secondary metabolite chemistry of different *Laurencia* species may prove to be a valuable chemotaxonomic tool at the species level, because the high degree of phenotypic variation frequently observed within a single given species complicates the taxonomy of this genus.[3]

As part of our continuing interest on the chemistry of the genus *Laurencia*,[4−7] in conjunction with the fact that *L. catarinensis* Cordeiro-Marino & Fujii has not been the subject of a previous study, we decided to investigate the chemical profile of this species. In this report, we describe the isolation and structure elucidation of seven new (**1**−**7**) and seven previously reported (**8**−**14**) halogenated metabolites and the assessment of their in vitro cytotoxicity against HT29, MCF7, and A431 tumor cell lines.

Results and Discussion

Specimens of the red alga *L. catarinensis*, collected in Ilha do Arvoredo, Brazil, were exhaustively extracted with a mixture of CH_2Cl_2/MeOH, and the organic extract was subsequently subjected to a series of chromatographic separations to allow for the isolation of compounds **1**−**14**.

Compound **1**, isolated as a colorless oil, had the molecular formula $C_{17}H_{27}Br_2ClO_4$, as deduced from the HRESIMS and NMR data. The mass spectrum exhibited pseudomolecular ion peaks



$[M + H]^+$ at *m*/*z* 489/491/493/495 with an isotopic ratio of 7:15:10:2, characteristic for the presence of one chlorine and two bromine atoms in the molecule. The ^{13}C NMR spectrum and the DEPT experiments revealed the presence of 17 carbon atoms corresponding to four quaternary carbons, five methines, three methylenes, and five methyls (Table 1). The chemical shifts of the seven carbons resonating at δ_C 52.4, 62.7, 69.4, 70.4, 70.7, 75.5, and 76.4 implied their direct bonding to oxygen or halogen atoms. The presence of an acetoxy group was suggested by a carbonyl carbon resonating at δ_C 169.6 and a singlet methyl resonating at δ_H 2.02. The ^1H NMR spectrum further included four singlet methyls (δ_H 1.12, 1.26, 1.29, and 1.73) and four deshielded methine protons (δ_H 3.53, 4.25, 4.41, and 5.40) (Table 2). With three degrees of unsaturation, the structure of **1** was determined as bicyclic. The fact that the molecule contained three halogen and four oxygen atoms (two of the latter participating in the acetoxy group), in conjunction with the presence of seven deshielded carbons, suggested an ether bridge in the carbocycle of **1**, a hypothesis that was further confirmed by the absorption at 1071 cm^{-1} observed in the IR spectrum. Analysis of the 2D NMR spectra allowed the assignment of the ^1H and ^{13}C chemical shifts that led to the structure determination of metabolite **1**. In particular, the COSY cross-peaks of H-2/H_2-3, H_2-3/H-4, H-4/H-5, and H-5/H_2-6, as well as the correlations of C-1, C-2, and C-6 with H_3-15 evident in the HMBC spectrum, established the cyclohexane ring. The HMBC correlations of C-4, C-7, and C-8 with H_3-14 and of C-10 and C-11 with both H_3-12 and H_3-13, in

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Table 1. ^{1}H NMR Data (400 MHz, $CDCl_3$) of Compounds **1**−**7**

position		**1** (*J* in Hz)		**2** (*J* in Hz)		**3** (*J* in Hz)		**4** (*J* in Hz)		**5** (*J* in Hz)		**6** (*J* in Hz)		**7** (*J* in Hz)
2		4.41, dd (11.3, 5.0)		4.25, dd (12.7, 3.9)		4.21, dd (12.3, 4.0)		4.38, dd (12.4, 4.3)		4.34, dd (12.8, 4.4)		4.34, dd (12.8, 4.1)		4.34, dd (12.4, 4.2)
3	ax	2.07, m	ax	2.28, dt (13.2, 12.7)	ax	2.11, m	ax	2.17, td (13.3, 12.4)	ax	1.80, td (12.8, 12.4)	ax	1.99, td (13.2, 12.8)	ax	1.91, dt (13.2, 12.4)
	eq	2.05, m	eq	1.94, m	eq	1.82, m	eq	2.27, m	eq	2.31, m	eq	2.42, m	eq	2.53, m
4		2.29, brd (11.4)		1.69, m		2.00, m		1.63, m		1.58, m		2.41, m		2.42, dddd (12.4, 12.4, 3.7, 3.3)
5		5.40, brs		4.50, brs		5.42, brs		5.35, brs	ax	1.34, m	ax	1.48, dddd (13.9, 13.9, 12.8, 3.3)	ax	1.56, dddd (13.9, 13.9, 12.4, 3.3)
									eq	1.88, m	eq	2.01, m	eq	1.89, dddd (13.9, 3.7, 3.7, 3.3)
6	ax	2.20, m	ax	2.11, m	ax	2.18, m	ax	2.15, m	ax	2.03, m	ax	2.13, ddd (13.9, 13.5, 3.7)	ax	2.14, td (13.9, 3.7)
	eq	2.69, dd (15.0, 2.9)	eq	2.57, dd (14.3, 3.3)	eq	2.73, dd (15.1, 3.1)	eq	2.70, dd (15.0, 3.3)	eq	2.40, dt (13.5, 3.3)	eq	2.47, ddd (13.5, 3.3, 2.9)	eq	2.48, ddd (13.9, 3.7, 3.3)
8		3.53, brs		4.73, brs		4.67, brs	ax	1.57, m	ax	1.25, m		5.88, s		5.88, s
							eq	1.48, dt (13.6, 3.9)	eq	2.06, m				
9	ax	2.51, ddd (13.9, 13.2, 2.3)	ax	2.54, m	ax	2.49, ddd (14.6, 13.2, 2.6)	ax	2.22, m	ax	2.34, m				
	eq	2.16, m	eq	2.24, ddd (14.6, 4.0, 3.7)	eq	2.25, dt (14.6, 3.9)	eq	2.08, m	eq	2.04, m				
10		4.25, dd (13.2, 3.8)		4.14, dd (13.2, 4.0)		4.08, dd (13.2, 3.9)		3.81, dd (12.6, 4.0)		4.16, dd (4.4, 3.3)				
11												1.62, s		1.61, s
12		1.26, s		1.36, s		1.30, s		1.26, s		1.28, s		1.73, s		1.73, s
13		1.29, s		1.42, s		1.34, s		1.37, s		1.35, s				
14		1.12, s		1.32, s		1.21, s		1.20, s		1.14, s				
15		1.73, s		1.88, s		1.74, s		1.75, s		1.68, s				
5-OAc		2.02, s				2.04, s		2.02, s						
8-OAc				2.13, s		2.16, s								
10-OMe												3.18, s		3.19, s
OH		2.39, brs		2.45, brs										

Table 2. ^{13}C NMR Data (50.3 MHz, $CDCl_3$) of Compounds **1**−**7**

position	**1**		**2**		**3**		**4**		**5**		**6**		**7**	
1	70.4	C	71.8	C	70.5	C	70.5	C	71.9	C	70.0	C	70.0	C
2	62.7	CH	63.5	CH	61.9	CH	62.9	CH	64.1	CH	60.5	CH	60.7	CH
3	32.4	CH_2	31.8	CH_2	32.5	CH_2	32.2	CH_2	36.4	CH_2	40.1	CH_2	39.3	CH_2
4	46.2	CH	48.9	CH	46.4	CH	53.7	CH	50.0	CH	36.9	CH	36.9	CH
5	69.4	CH	67.7	CH	68.9	CH	68.8	CH	23.5	CH_2	28.2	CH_2	29.1	CH_2
6	46.4	CH_2	49.5	CH_2	46.4	CH_2	46.4	CH_2	42.8	CH_2	42.3	CH_2	42.2	CH_2
7	75.5	C	77.2	C	75.4	C	74.5	C	74.2	C	169.0	C	169.1	C
8	70.7	CH	73.9	CH	72.5	CH	36.3	CH_2	27.9	CH_2	118.0	CH	118.0	CH
9	35.7	CH_2	33.0	CH_2	32.2	CH_2	27.9	CH_2	26.3	CH_2	170.6	C	170.7	C
10	52.4	CH	51.6	CH	51.9	CH	57.6	CH	59.6	CH	109.0	C	109.0	C
11	76.4	C	76.9	C	75.8	C	75.6	C	73.6	C	22.6	CH_3	22.6	CH_3
12	30.8	CH_3	30.8	CH_3	30.8	CH_3	31.2	CH_3	31.5	CH_3	23.9	CH_3	23.9	CH_3
13	23.9	CH_3	24.0	CH_3	24.0	CH_3	23.8	CH_3	28.3	CH_3				
14	21.4	CH_3	23.5	CH_3	21.4	CH_3	23.1	CH_3	24.7	CH_3				
15	27.3	CH_3	28.1	CH_3	27.3	CH_3	27.3	CH_3	24.1	CH_3				
5-OAc	169.6	C			169.4	C	169.4	C						
5-OAc	21.3	CH_3			21.1	CH_3	21.4	CH_3						
8-OAc			169.7	C	169.7	C								
8-OAc			21.2	CH_3	21.0	CH_3								
10-OMe											51.0	CH_3	51.0	CH_3

combination with the interactions of H-8/H_2-9 and H_2-9/H-10 displayed in the COSY spectrum, suggested the existence of a carbon−carbon single bond between C-4 and C-7 and the position of the *gem*-dimethyl group on C-11. The remaining oxygen atom was placed between C-7 and C-11, completing the tetrahydropyran ring. Comparison of the spectroscopic data of metabolite **1** with those of previously reported compounds bearing similar structural features indicated that it was the 5-acetoxy derivative of the halogenated sesquiterpene caespitol (**8**).[8−11] The relative configuration of **1** was proposed on the basis of the measured coupling constants and the observed enhancements in the NOESY spectrum. The equatorial orientation of the bromine on C-2 was indicated by the coupling constants of the halomethine proton H-2 (J = 11.3, 5.0 Hz), whereas the chemical shifts of C-1 (δ_C 70.4) and C-2 (δ_C 62.7) were in accord with the expected values for vicinal carbons bearing diequatorial halogens in cyclohexane rings.[10] The fact that H-5 appeared as a broad singlet implied its equatorial orientation. Furthermore, the fact that C-15 resonated at lower fields in **1** (δ_C 27.3), in comparison to **8** (δ_C 24.1), could be explained by the deshielding effect of the axially oriented acetoxy group at C-5, as in the case of deodactol and isodeodactol.[12,13] The NOE enhancement between methines H-2 and H-4 implied their diaxial orientation, thus concluding the relative configuration of the stereogenic centers in the cyclohexane ring. In the tetrahydropyran ring, the intense NOE enhancement of H_3-13/H_3-14, in conjunction with the coupling constants of H-8 (brs) and H-10 (J = 13.2, 3.8 Hz), clarified the relative configuration at C-7, C-8, and C-10.

The absolute configuration of **1** was determined by application of the modified Mosher's method.[14] When **1** was treated with (*R*)- and (*S*)-MTPA chloride, the secondary hydroxy group at C-8 reacted to give the (*S*)- and (*R*)-MTPA derivatives, respectively. The ^1H NMR chemical shifts of the MTPA derivatives of **1** were assigned by analyses of ^1H, COSY, and 1D NOE NMR spectra. The calculation of the $\Delta\delta_{S-R}$ values, shown in Figure 1, defined the

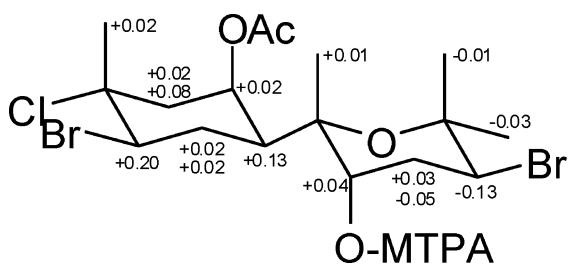

Figure 1. $\Delta\delta_{S-R}$ values (ppm) for the MTPA derivatives of **1** in $CDCl_3$.

absolute configuration of C-8 as *S* and subsequently, on the basis of its relative configuration, established the absolute stereochemistry of **1** as depicted. On the basis of the above-mentioned data, metabolite **1** was identified as (5*S*)-5-acetoxycaespitol.

Compound **2**, isolated as a colorless oil, exhibited the same molecular formula as **1**, according to the HRESIMS and NMR data. The NMR spectroscopic features of **2** (Tables 1 and 2) showed close resemblance with those of metabolite **1**, suggesting the two to be positional isomers. In particular, in the ^1H NMR spectrum of **2** five singlet methyls (δ_H 1.32, 1.36, 1.42, 1.88, and 2.13) and four deshielded methine protons (δ_H 4.14, 4.25, 4.50, and 4.73) were evident, while the ^{13}C NMR spectrum and DEPT experiments revealed the presence of four quaternary carbons, five methines, three methylenes, and five methyls. The two methines resonating at δ_H 3.53 and 5.40 in **1** were shifted to δ_H 4.73 and 4.50, respectively, in **2**, implying that the positions of the hydroxy and the acetoxy groups were interchanged. This hypothesis was verified by the ^1H and ^{13}C chemical shift assignment and the homo- and heteronuclear correlations displayed in the COSY and HMBC spectra. The equatorial orientation of the bromine at C-10 and the axial orientation of the acetoxy group at C-8 in the tetrahydropyran ring were deduced on the basis of the coupling constants of H-8 (brs) and H-10 (J = 13.2, 4.0 Hz), while the observed NOE enhancement between H_3-13 and H_3-14 revealed their diaxial orientation. In the cyclohexane ring, the diequatorial orientation of the chlorine and the bromine atoms was proposed on the basis of the chemical shifts of the halogen-bearing carbons C-1 and C-2, whereas the coupling constants of H-2 (J = 12.7, 3.9 Hz) and H-5 (brs), in combination with the NOE enhancement observed between H-2 and H-4, suggested the same relative configuration at C-2, C-4, and C-5 as in **1**. Therefore, compound **2** was identified as (5*S*)-5-hydroxyacetylcaespitol.

Compound **3**, isolated as a colorless oil, had the molecular formula $C_{19}H_{29}Br_2ClO_5$, as determined from the HRESIMS and NMR data. The NMR spectroscopic features of **3** (Tables 1 and 2) were very similar to those of metabolites **1** and **2**, with the most prominent differences being the presence of two ester carbonyls and two acetoxy methyls in the ^{13}C and ^1H NMR spectra, respectively. The shift of H-5 and H-8 to lower fields at 5.42 and 4.67 ppm, respectively, was indicative of the acetylation of the corresponding hydroxy groups. The correlations observed in the homo- and heteronuclear experiments supported the proposed structure of **3** as the acetyl derivative of **1** or **2**. The relative configuration, as deduced from the coupling constants and NOE

enhancements, was found to be the same as that of metabolites **1** and **2**. Thus, compound **3** was identified as (5*S*)-5-acetoxyacetylcaespitol.

Compound **4**, obtained as a colorless oil, exhibited the molecular formula $C_{17}H_{27}Br_2ClO_3$, as calculated from the HRESIMS measurements. The ^{13}C NMR spectrum and the DEPT experiments showed the presence of four quaternary, four methine, four methylene, and five methyl carbon atoms, whereas in the ^1H NMR spectrum of **4** five singlet methyls (δ_H 1.20, 1.26, 1.37, 1.75, and 2.02) and three deshielded methines (δ_H 3.81, 4.38, and 5.35) were evident. Analysis of the spectroscopic data of **4** (Tables 1 and 2) showed a high degree of similarity with metabolite **1**. In agreement with the molecular formula, it was clear that the difference was the absence of the hydroxy group. The correlations displayed in the 2D NMR spectra supported the proposed structure, while the analysis of the coupling constants and the NOE spectrum verified the relative configuration, thus identifying **4** as (5*S*)-5-acetoxydeoxycaespitol.

Compound **5** was obtained as a colorless oil that, similarly to the previously described metabolites, showed a molecular ion cluster with an isotopic ratio characteristic for the presence of one chlorine and two bromine atoms. The HRESIMS data indicated the molecular formula $C_{15}H_{25}Br_2ClO$. The ^{13}C NMR and DEPT spectra revealed the presence of four methyl groups, five methylenes, three methines, and three quaternary carbon atoms (Table 1). In contrast to **1**−**4**, the ^1H NMR spectrum of metabolite **5** did not contain any low-field signals attributable to oxygenated methines (Table 2). Another notable difference observed was the measured coupling constants of the signal assigned to the halogenated methine H-10 at δ_H 4.16 (J = 4.4, 3.3 Hz). The correlations obtained from the 2D NMR spectra confirmed the planar structure of compound **5**. The relative configuration at C-1, C-2, and C-4 in the cyclohexane ring was the same as in **1**−**4**, as indicated by the chemical shifts of C-1 and C-2 and the NOE enhancement observed between H-2 and H-4. On the contrary, in the tetrahydropyran ring the small values of the coupling constants of H-10 (J = 4.4, 3.3 Hz) implied inversion of the configuration at C-10. The axial orientation of the bromine atom at C-10 was further supported by the downfield shifts of C-10 (δ_C 59.6) and C-13 (δ_C 28.3), as well as of H-10 (δ_H 4.16), in comparison to the chemical shifts observed for metabolite **4** (δ_C 57.6 and 23.8, δ_H 3.81, respectively). Compound **5**, identified as 10-*epi*-deoxycaespitol, represents the first caespitol-related natural product with an axial bromine at C-10. The absolute configurations of metabolites **2**−**5** were not determined, but on the basis of biogenetic considerations they are expected to be identical to that of **1**.

Compounds **6** and **7**, obtained in the form of colorless crystals, were isolated through HPLC as two distinct peaks. Both metabolites possessed the molecular formula $C_{13}H_{18}BrClO_3$, as deduced from HRESIMS measurements, and exhibited very similar NMR spectroscopic data. The ^{13}C NMR spectra and DEPT experiments of **6** and **7** indicated the presence of three methyls, three methylenes, three methines, and four quaternary carbon atoms, among which were two olefinic carbons (one tertiary and one quaternary) and a carbonyl (Table 1). The presence of an olefinic proton, a methoxy group, and two aliphatic methyls appearing as singlets was obvious in the ^1H NMR spectra of both compounds (Table 2). Taking into account the above data and the four degrees of unsaturation, **6** and **7** were assumed to be bicyclic. Analyses of the 2D NMR spectra resulted in the establishment of the same planar structure, suggesting that the two compounds were stereoisomers. Comparison with previously reported compounds indicated that either **6** or **7** was the 10-*O*-methyl derivative of furocaespitanelactol (**13**) and the other its epimer at C-10.[15] As indicated from the chemical shifts of C-1 and C-2 and the NOE enhancement of H-2/H-4, the relative configuration of the stereogenic centers C-1, C-2, and C-4 in the cyclohexane ring was retained as in metabolites **1**−**5**, further supporting that **6** and **7** were epimers at C-10. In the 1D NOE experiments, irradiation of the methoxy group in **6** led to the enhancement of H_3-12, whereas irradiation of H_3-11 led to the enhancement of H-2, implying the *R** configuration for C-10. On the contrary, irradiation of the olefinic proton H-8 in **7** led to the enhancement of the methoxy group, confirming the different orientation of the latter. According to the proposed configuration, the orientation of the methoxy group in **6** could explain the observed shielding effects on the chemical shifts of H-3_{eq} and H-5_{ax}, as well as the deshielding effects on the chemical shifts of H-3_{ax} and H-5_{eq}, when compared to those of **7**. Single-crystal X-ray diffraction analysis of **6** (Figure 2) verified the proposed structure and relative configuration.[16] Therefore, compounds **6** and **7** were identified as (10*R**)- and (10*S**)-10-*O*-methylfurocaespitanelactol, respectively.

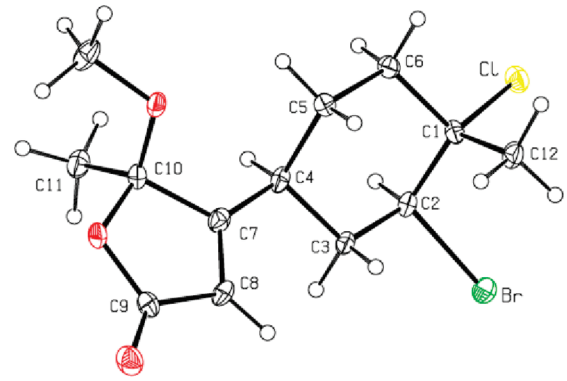

Figure 2. ORTEP drawing of compound **6**. Displacement ellipsoids are drawn at the 30% probability level. The drawing does not imply absolute configuration.

Table 3. In Vitro Cytotoxicity[a] of Compounds **1**−**14**

compound	HT29	MCF7	A431
1	12.4 ± 0.5	11.7 ± 0.4	13.1 ± 0.7
2	70.4 ± 6.5	73.6 ± 5.3	72.1 ± 9.1
3	inactive[b]	inactive[b]	inactive[b]
4	48.8 ± 1.9	52.4 ± 2.0	54.9 ± 3.4
5	inactive[b]	inactive[b]	inactive[b]
6	inactive[b]	inactive[b]	inactive[b]
7	85.6 ± 11.4	89.0 ± 9.6	91.7 ± 10.3
8	7.6 ± 0.4	9.7 ± 0.6	10.2 ± 0.8
9	16.1 ± 0.8	17.6 ± 1.2	17.4 ± 0.9
10	12.5 ± 0.6	11.2 ± 0.9	13.1 ± 1.2
11	inactive[b]	inactive[b]	inactive[b]
12	27.3 ± 1.2	31.7 ± 2.6	30.7 ± 4.1
13	inactive[b]	inactive[b]	inactive[b]
14	18.9 ± 1.4	19.7 ± 0.8	21.6 ± 0.6
miltefosine	48.6 ± 4.0	42.3 ± 3.8	53.7 ± 6.2

[a] Expressed as IC_{50} (in μM). [b] IC_{50} > 100 μM.

In order to investigate the possibility of compounds **6** and **7** being artifacts of the extraction and isolation processes, metabolite **13** (1.6 mg) was treated with silica gel (200 mg) in MeOH (3 mL) and left under constant stirring at room temperature for 4 days. After filtration of the mixture and evaporation of the solvent, the residue was examined by ^1H NMR and TLC (using **6** and **7** as markers) but did not show any conversion of **13** to either **6** or **7**. However, the natural origin of metabolites **6**, **7**, and **13** in the investigated organism cannot be unambiguously proposed, because these butenolides could potentially be artifacts of oxidation of a substituted furan nucleus by singlet oxygen.[17]

In addition to the new metabolites **1**−**7**, seven known natural products were isolated and identified as caespitol (**8**),[8−11] acetylcaespitol (**9**),[11] deoxycaespitol (**10**),[18] isocaespitol (**11**),[9,10] deoxyisocaespitol (**12**),[19] furocaespitanelactol (**13**),[15] and caespitenone (**14**)[11] by comparison of their spectroscopic and physical characteristics with those reported in the literature.

Metabolites **1**−**14** were evaluated for their in vitro cytotoxic activity against three human tumor cell lines (HT29, MCF7, and

A431). The results of the cytotoxicity assessment of the tested compounds after 24 h of incubation are given in Table 3. It is worth noting that none of the metabolites showed differential cytotoxicity against any of the tested cell lines. Compounds **1**, **8**−**10**, and **14** were found to be considerably more active among all evaluated metabolites, exhibiting activity with IC_{50} values lower than 20 μM, whereas metabolites **2**−**7** and **11**−**13** displayed weak or no activity. Two of the most active compounds (**1** and **8**) possessed a free hydroxy group, while **3** and **5**−**7**, which did not possess any free hydroxy groups, were inactive. Contrary to this trend, metabolites **9**, **10**, and **14**, which were lacking free hydroxy groups, were among the most active ones, and compound **13**, possessing a free hydroxy group, was inactive. Therefore, comparison of the observed levels of activity, taking into account the structural similarities and differences of compounds **1**−**14**, could not lead to safe conclusions on the specific features that influence their cytotoxicity. Even though the panel of the tested compounds is rather small, it seems that the cytotoxic activity cannot be correlated with the presence or absence of specific functional groups, and it is probably influenced by a combination of factors, including the overall three-dimensional structure of the molecules and the spatial orientation of their substituents. In favor of this assumption is the marked difference in activity observed for the isomeric metabolites **5** and **12**, which differ only in the position of the halogens and the orientation of the substituents at C-1, C-2, and C-10. Comparison of the activity of compounds **1**, **8**, and **10** with that of miltefosine (used as positive control) revealed a surprisingly high and acute cytotoxic effect, which was more pronounced for **8** and **10** with increasing exposure duration (data not shown). These promising results necessitate further studies aiming at the evaluation of their cytotoxic activity in a broader cell line panel.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter with a 1 dm cell. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained on a Paragon 500 Perkin-Elmer spectrometer. NMR spectra were recorded on Bruker AC 200 and Bruker DRX 400 spectrometers. Chemical shifts are given on the δ (ppm) scale using TMS as internal standard. The 2D experiments (HMQC, HMBC, COSY, NOESY) were performed using standard Bruker pulse sequences. High-resolution ESI mass spectra were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, IN. Low-resolution EI and CI mass spectra were recorded on a Thermo Electron Corporation DSQ mass spectrometer using a direct exposure probe and methane as the CI reagent gas. Column chromatography was performed using Kieselgel 60 (Merck). HPLC separations were conducted using a Pharmacia LKB 2252 liquid chromatography pump equipped with a Shodex 102 refractive index detector, using a Supelcosil SPLC-Si (Supelco, 25 cm × 10 mm) column. TLC was performed using Kieselgel 60 F_{254} (Merck aluminum support plates), and spots were detected after spraying with 15% H_2SO_4 in MeOH reagent and heating at 100 °C for 1 min.

Plant Material. Specimens of *Laurencia catarinensis* were hand-collected in Ilha do Arvoredo, Santa Catarina, Brazil, at a depth of 1−2 m, in February 2008. A voucher specimen of the alga has been deposited at the Herbarium of the Department of Botany, Federal University of Santa Catarina (FLOR 14516).

Extraction and Isolation. Specimens of the air-dried alga (400 g) were exhaustively extracted with CH_2Cl_2/MeOH (2:1) at room temperature. Evaporation of the solvent in vacuo afforded a green oily residue (6.9 g), which was subjected to vacuum column chromatography on silica gel, using cyclohexane with increasing amounts of EtOAc, followed by EtOAc with increasing amounts of MeOH as the mobile phase, to afford 17 fractions (A−Q). Fraction E (15% EtOAc, 1.1 g) was further fractionated by gravity column chromatography on silica gel, using cyclohexane with increasing amounts of EtOAc as the mobile phase, to yield 24 fractions (E1−E24). Fraction E2 (2% EtOAc, 60.6 mg) was purified by normal-phase HPLC, using cyclohexane/EtOAc (99:1) as eluant, to afford compounds **5** (2.6 mg), **10** (9.0 mg), and **12** (1.5 mg). Fractions E6 (2% EtOAc, 2.7 mg) and E7 (2% EtOAc, 31.1 mg) were subjected separately to normal-phase HPLC, using cyclohexane/EtOAc (98:2) as eluant, to yield compound **9** (2.1 mg). Fractions E8 (2% EtOAc, 7.3 mg) and E9 (4% EtOAc, 13.7 mg) were purified separately by normal-phase HPLC, using cyclohexane/EtOAc (95:5) as eluant, to afford compound **11** (15.2 mg). Fractions E12 (4% EtOAc, 13.0 mg) and E13 (6% EtOAc, 17.0 mg) were subjected separately to normal-phase HPLC, using cyclohexane/EtOAc (95:5) as eluant, to yield compound **4** (20.5 mg). Fractions E14 (6% EtOAc, 210.9 mg), E15 (6% EtOAc, 32.1 mg), and E16 (6% EtOAc, 48.0 mg) were purified separately by normal-phase HPLC, using cyclohexane/EtOAc (95:5) as eluant, to afford compound **8** (220.2 mg). Fraction E18 (8% EtOAc, 50.6 mg) was subjected to normal-phase HPLC, using cyclohexane/EtOAc (95:5) as eluant, to yield compound **14** (5.2 mg). Fraction E20 (8% EtOAc, 24.4 mg) was subjected to normal-phase HPLC, using cyclohexane/EtOAc (93:7) as eluant, to yield compound **3** (1.3 mg). Fractions E21 (10% EtOAc, 88.3 mg) and E22 (10% EtOAc, 54.8 mg) were purified separately by normal-phase HPLC, using cyclohexane/EtOAc (93:7) as eluant, to afford compound **1** (123.3 mg). Fraction E24 (15% EtOAc, 119.7 mg) was subjected to normal-phase HPLC, using cyclohexane/EtOAc (91:9) as eluant, to yield compound **2** (3.0 mg). Fraction G (25% EtOAc, 127.4 mg) was purified by normal-phase HPLC, using cyclohexane/EtOAc (90:10) as eluant, to afford compounds **6** (10.3 mg) and **7** (12.6 mg). Finally, fraction I (30% EtOAc, 38.6 mg) was subjected to normal-phase HPLC, using cyclohexane/EtOAc (80:20) as eluant, to yield compound **13** (5.9 mg).

(5*S*)-5-Acetoxycaespitol (1): colorless oil; $[\alpha]^{20}_D$ +50.5 (*c* 0.41, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243.4 (2.08) nm; IR (thin film) ν_{max} 3502, 2986, 1734, 1458, 1384, 1239, 1071, 739 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)CIMS (CH_4) *m*/*z* (rel int %) 489/491/493/495 (7:15:10:2), 429/431/433/435 (37:74:50:12), 411/413/415/417 (47:84:57:14), 393/395 (29:27), 349/351 (23:25), 331/333 (25:27), 313/315 (100:83), 295/297 (38:36), 251/253 (33:36), 221/223 (64:61), 177/179 (40:37), 119 (30), 99 (30), 84 (38), 69 (12); HRESIMS *m*/*z* 510.9838 $[M + Na]^+$ (calcd for $C_{17}H_{27}Br_2ClNaO_4$, 510.9862).

(5*S*)-5-Hydroxyacetylcaespitol (2): colorless oil; $[\alpha]^{20}_D$ +9.2 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243.4 (2.51) nm; IR (thin film) ν_{max} 3502, 2939, 1734, 1444, 1374, 1239, 1122, 1034 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)CIMS (CH_4) *m*/*z* (rel int %) 489/491/493/495 (1:3:2:0.5), 471/473/475/477 (4:7:6:1), 453/455/457 (3:6:3), 411/413 (6:6), 393/395 (6:6), 379 (16), 349/351 (28:30), 313/315 (45:47), 295/297 (20:19), 263 (92), 251 (34), 233 (62), 215 (50), 199 (35), 161 (31), 141 (100), 123 (64), 93 (47), 81 (49), 71 (34), 61 (21); HRESIMS *m*/*z* 489.0017 $[M + H]^+$ (calcd for $C_{17}H_{28}Br_2ClO_4$, 489.0043).

(5*S*)-5-Acetoxyacetylcaespitol (3): colorless oil; $[\alpha]^{20}_D$ +25 (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242.6 (2.58) nm; IR (thin film) ν_{max} 2978, 1740, 1734, 1374, 1230, 1118 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)CIMS (CH_4) *m*/*z* (rel int %) 392 (4), 353/355/357 (4:7:4), 313/315/317 (11:18:12), 295/297 (13:14), 263/265 (12:12), 251 (19), 233/235 (12:14), 214 (90), 199 (76), 171 (17), 135 (31), 123 (62), 91 (28), 81 (19), 61 (100); HRESIMS *m*/*z* 531.0124 $[M + H]^+$ (calcd for $C_{19}H_{30}Br_2ClO_5$, 531.0149).

(5*S*)-5-Acetoxydeoxycaespitol (4): colorless oil; $[\alpha]^{20}_D$ +36.3 (*c* 0.49, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246.8 (2.30) nm; IR (thin film) ν_{max} 2967, 1739, 1449, 1379, 1239, 1015, 744 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)CIMS (CH_4) *m*/*z* (rel int %) 413/415/417 (5:9:6), 333/335/337 (13:20:11), 315/317 (10:10), 297/299 (6:6), 253 (7), 217 (20), 205/207 (100:97), 199 (14), 125 (48), 93 (41), 61 (31); HRESIMS *m*/*z* 470.9895 $[M - H]^+$ (calcd for $C_{17}H_{26}Br_2ClO_3$, 470.9937).

10-*epi*-Deoxycaespitol (5): colorless oil; $[\alpha]^{20}_D$ +24.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243.0 (2.56) nm; IR (thin film) ν_{max} 2939, 2854, 1458, 1379, 1230, 1118, 1006, 978 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS (70 eV) *m*/*z* (rel int %) 399/401/403 (1:2:1), 356/358/360 (1:2:1), 250/252 (4:5), 205/207 (100:96), 135 (20), 125 (38), 107 (47), 93 (27), 55 (17); HRESIMS *m*/*z* 414.9998 $[M + H]^+$ (calcd for $C_{15}H_{26}Br_2ClO$, 415.0039).

(10*R)-10-*O*-Methylfurocaespitanelactol (6):** colorless crystals; $[\alpha]^{20}_D$ +14 (*c* 0.09, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242.8 (2.43) nm; IR (thin film) ν_{max} 1767, 1465, 1375, 1094 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)CIMS (CH_4) *m*/*z* (rel int %) 337/339/341 (61:75:17), 321/323 (33:36), 305/307 (44:47), 293/295 (27:30), 269/271 (49:50). 239 (31), 225/227 (100:38), 183/185 (91:31), 161 (25), 141 (35), 119 (22); HRESIMS *m*/*z* 337.0190 $[M + H]^+$ (calcd for $C_{13}H_{19}BrClO_3$, 337.0206).

(10*S)-10-*O*-Methylfurocaespitanelactol (7):** colorless crystals; $[\alpha]^{20}_{D}$ +61.3 (*c* 0.16, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 244.6 (2.52) nm; IR (thin film) ν_{max} 1762, 1463, 1374, 1103 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; (+)CIMS (CH_4) *m*/*z* (rel int %) 337/339/341 (65:87:23), 321/323 (35:37), 305/307 (33:36), 293/295 (23:26), 269/271 (34:36), 238 (4), 225/227 (83:31), 183/185 (100:36), 161 (34), 147 (37), 119 (36), 96 (50); HRESIMS *m*/*z* 337.0195 $[M + H]^+$ (calcd for $C_{13}H_{19}BrClO_3$, 337.0206).

Preparation of MTPA Derivatives of 1. Compound **1** (6.0 mg) was treated with (*R*)-MTPA chloride (7 μL) in freshly distilled dry pyridine (1 mL) and left under constant stirring at room temperature for 18 h. The reaction was quenched by the addition of H_2O (3 mL) and CH_2Cl_2 (3 mL), and the mixture was partitioned between the aqueous and the organic layer. After evaporation of the organic layer in vacuo, the residue was purified by normal-phase HPLC, using cyclohexane/EtOAc (97:3) as eluant, to give the (*S*)-MTPA derivative (1.6 mg). The (*R*)-MTPA derivative (2.2 mg) was prepared with (*S*)-MTPA chloride and purified in the same manner.

(*R*)-MTPA Derivative of 1: ^1H NMR (400 MHz, $CDCl_3$) δ 7.56−7.48 (m, 5H, Ar-H), 5.29 (brs, 1H, H-5), 4.77 (brs, 1H, H-8), 3.92 (brd, 12.9 Hz, 1H, H-10), 3.58 (brd, 11.4 Hz, 1H, H-2), 3.45 (s, 3H, OMe), 2.60 (dd, 15.0, 3.0 Hz, 1H, H-6_{ax}), 2.50 (t, 14.1 Hz, 1H, H-9_{ax}), 2.35 (dt, 14.6, 3.6 Hz, 1H, H-9_{eq}), 2.02−1.98 (m, 1H, H-3_{ax}), 2.00 (s, 3H, OAc), 1.80 (brd, 14.5 Hz, 1H, H-6_{eq}), 1.66 (s, 3H, H-15), 1.64 (brd, 13.3 Hz, 1H, H-4), 1.62−1.58 (m, 1H, H-3_{eq}), 1.31 (s, 3H, H-13), 1.24 (s, 3H, H-12), 1.20 (s, 3H, H-14).

(*S*)-MTPA Derivative of 1: ^1H NMR (400 MHz, $CDCl_3$) δ 7.54−7.46 (m, 5H, Ar-H), 5.31 (brs, 1H, H-5), 4.81 (dd, 3.8, 2.0 Hz, 1H, H-8), 3.79 (dd, 13.2, 4.0 Hz, 1H, H-10), 3.78 (dd, 12.6, 4.0 Hz, 1H, H-2), 3.56 (s, 3H, OMe), 2.62 (dd, 15.1, 3.1 Hz, 1H, H-6_{ax}), 2.55−2.51 (m, 1H, H-9_{ax}), 2.30 (dt, 15.0, 3.8 Hz, 1H, H-9_{eq}), 2.04−2.00 (m, 1H, H-3_{ax}), 2.00 (s, 3H, OAc), 1.88 (dd, 15.1, 2.5 Hz, 1H, H-6_{eq}), 1.77 (dt, 13.3, 2.3 Hz, 1H, H-4), 1.68 (s, 3H, H-15), 1.62 (brd, 12.4 Hz, 1H, H-3_{eq}), 1.30 (s, 3H, H-13), 1.21 (s, 6H, H-12 and H-14).

Single-Crystal X-ray Analysis. Single-crystal X-ray diffraction data were collected at 120 K on a Nonius Kappa CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) using the Nonius Collect software. After the initial corrections and data reduction, intensities of reflections were used to solve (by direct methods) and refine the structures (on F^2) using the WINGX program. A weighting scheme based upon $P = [F_o^2 + 2F_o^2]/3$ was employed. All the hydrogen atoms were located from difference maps and included in the refinements as riding. Compound **6** crystallized after slow evaporation of a saturated solution of MeOH as colorless blocks in the monoclinic system, space group $P2_1$ with a = 6.0017(5) Å, b = 11.9713(6) Å, c = 9.5760(8) Å, β = 93.375(3)°, V = 686.82 Å^3, Z = 2, d_{calc} = 1.63 g/cm^3. The numbers of measured, independent, and observed parameters were 9530, 3090, and 2004, respectively, with an initial R = 0.082. The refined structural model converged to a final R = 0.054 for observed reflections [$F_o > 4\sigma(F_o)$] and R_1 = 0.1153, wR_2 = 0.1326, S = 1.03 for all data with 166 parameters. The Flack parameter was 0.03(11).

Determination of Cytotoxicity. HT29 (human colorectal adenocarcinoma), MCF7 (human mammary adenocarcinoma), and A431 (human epidermoid carcinoma) cells were routinely cultured in Dulbecco's minimal essential medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% fetal bovine serum, in an environment of 5% CO_2 and 85% humidity at 37 °C, and they were subcultured using a trypsin 0.25%−EDTA 0.02% solution. Cells were plated in 96-well flat-bottomed microplates at a density of 1 × 10^5 cells/mL (100 μL/well), and the compounds were added, appropriately diluted in DMSO, 24 h later. Cells were exposed to various concentrations of the compounds for 24 h. The cytotoxicity was determined with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye reduction assay. Briefly, after incubation with the test compounds, MTT solution (5 mg/mL in PBS) was added (20 μL/well). Plates were further incubated for 4 h at 37 °C, and the formazan crystals formed were dissolved by adding 100 μL/well of 0.1 N HCl in 2-propanol. Absorption was measured by an enzyme-linked immunosorbant assay (ELISA) reader at 545 nm, with reference filter at 690 nm. For each concentration at least nine wells were used from three separate experiments. A 100 μL amount of culture medium supplemented with the same amount of MTT solution and solvent was used as blank solution. Data obtained were presented as IC_{50} (μM), which is the concentration of the compound where 100 × $(A_0 - A)/A_0$ = 50. In this formula, A is the optical density of the wells after 24 h of exposure to the test compound and A_0 is the optical density of the control wells. Miltefosine, an antiprotozoal drug, which was originally developed as an antineoplastic, was used as positive control. All data were expressed as mean ± SD. Statistical significance of differences was evaluated with one-way ANOVA followed by the Student−Newman−Keuls' test. Probability values < 5% were considered significant. Culture media and antibiotics were from Biochrom KG (Berlin, Germany). All other chemicals were from Sigma-Aldrich.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of the new compounds **1**−**7** and CIF data for the crystal structure of metabolite **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Crystallographic data (excluding structure factors) for compound **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 747674. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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