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Halogenated C_{15} Acetogenin Analogues of Obtusallene III from a *Laurenciella* sp. Collected in Corsica

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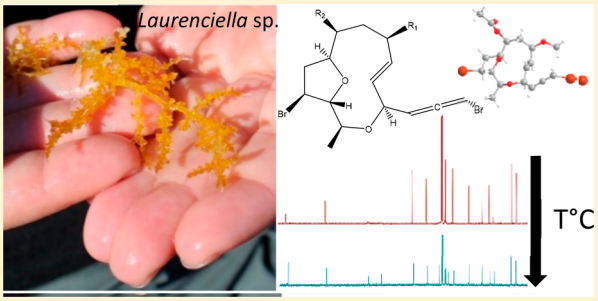
Supporting Information

ABSTRACT: NMR chemical profiling of a *Laurenciella* sp. using a computerized method developed in our laboratory resulted in the identification of five new compounds (**1**−**5**) and 17 known compounds, among which 3-(*E*)-laurenyne represented by far the most abundant metabolite. Compounds **1** to **5** were isolated and fully characterized by detailed spectroscopic analysis. The absolute configuration and structural features of compound **1** were determined by single-crystal X-ray diffraction analysis. Compounds **1** to **4** are 12-membered cyclic ether acetogenins that are present in solution as interconverting conformers exhibiting an (a*R*) configuration of the bromoallene unit together with an *S* configuration at C-4. Among these, compound **3** is the first obtusallene derivative with bromine substituents at both the C-7 and C-12 positions. Compound **5** is an acetogenin bearing a [5.5.1]bicyclotridecane ring system. A plausible biosynthetic route to **1**−**4** is proposed.



Macroalgae of the tribe Laurencieae (family Rhodomelaceae, order Ceramiales) are widely distributed in tropical areas and temperate seas and oceans. *Laurencieae* species produce a tremendous variety of secondary metabolites from various compound classes, many of which bear uncommon carbon skeletons.[1] Compounds biosynthesized by *Laurencia* species include sesquiterpenes, diterpenes, triterpenes, and C_{15} acetogenins.[1] C_{15} acetogenins described from *Laurencia* species are mainly oxepanes and *O*-bridged 12-membered cyclic ethers.[2] In the latter, obtusallene derivatives isolated from *Laurencia obtusa* have been extensively investigated.[3] Obtusallenes I, II, III, and IV and derivatives bearing an *O*-bridged 12-membered ring have been shown to occur as temperature-dependent conformers attributable to a major conformer in equilibrium with a minor conformer by 180° flipping of the *trans* olefinic group.[3] A hypothetical biosynthetic pathway from the epoxy fatty acid derivative of the *Z*,*Z*,*Z*-hexadeca-4,7,10-trienoic acid to obtusallenes via multiple electrophilic brominations was described by Braddock.[4]

Recently, screening of the *Laurencia* complex based on molecular phylogeny revealed that the genus *Laurenciella*, previously known from the Canary Islands (Atlantic Ocean), is also present in the Mediterranean Sea.[5,6] A research group from the Canary Islands reported the identification of compounds isolated from *Laurencia marilzae*, a synonym of the taxonomically accepted name *Laurenciella marilzae*.[7] They elucidated the structures of C_{15} acetogenins belonging to two different categories of obtusallenes: obtusallene IV derivatives and marilzabicycloallenes.[7−9]

In the present study, specimens of *Laurenciella* sp. collected from the Ajaccio Bay in Corsica (France) were investigated. The isolation and structure elucidation of five new C_{15} acetogenins as well as the identification of 17 known compounds of this species are described. A biogenetic pathway leading to the new obtusallenes **1**−**4** is also discussed.

RESULTS AND DISCUSSION

Specimens of the red alga *Laurenciella* sp., collected in Ajaccio Bay, Corsica, France, were air-dried, frozen with liquid nitrogen, reduced to powder, and then extracted with EtOAc at room temperature. The organic extract was subjected to a series of chromatographic fractionations to facilitate the isolation of compounds **1**−**5**. The extract and all the fractions were subsequently analyzed by ^{13}C NMR spectroscopy, using a computerized method developed in our laboratory.[10−12] This technique relies on the identification of individual components within crude mixtures or chromatographic fractions by comparison of ^{13}C NMR signals with those of reference spectra present in a library.

Structural Elucidation of Compounds 1−5. The elemental composition of **1**, obtained as an optically active white powder from the EtOAc extract, was deduced to be $C_{18}H_{24}Br_2O_5$ based on the HRESIMS and NMR data. Signals observed in the ^1H and ^{13}C NMR spectra of **1** exhibited resonances for a bromoallene unit [δ_C 202.1 (C), δ_C 101.0

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Table 1. ^{13}C NMR (125.5 MHz, $CDCl_3$) Data for Major Conformers of Compounds 1−4 at 20 and −40 °C

		20 °C				−40 °C			
pos	type	1	2	3	4	1	2	3	4
1	CH	74.1	74.1	74.3	74.1	74.4	74.2	74.5	73.4
2	C	202.1	202.1	202.3	202.0	201.6	201.7	201.8	201.7
3	CH	101.0	100.7	100.3	100.9	100.2	99.8	99.4	99.8
4	CH	n.o.[a]	n.o.	n.o.	n.o.	79.1	78.8	78.4	79.2
5	CH	n.o.	n.o.	n.o.	n.o.	136.6	135.9	136.6	135.6
6	CH	n.o.	n.o.	n.o.	n.o.	126.4	128.2	126.1	128.5
7	CH	n.o.	n.o.	n.o.	n.o.	71.9	64.1	50.5	66.6
8	CH_2	35.8	37.8	38.7	41.4	34.8	36.8	37.6	40.1
9	CH	68.3	68.1	68.5	67.8	67.7	67.5	68.0	64.5
10	CH	74.5	74.4	73.9	75.7	73.9	73.8	73.4	74.4
11	CH_2	39.1	39.2	39.1	39.7	38.5	38.5	38.5	37.2
12	CH	44.9	44.6	44.4	44.9	44.4	44.3	44.1	44.7
13	CH	89.6	89.6	89.7	90.2	88.9	88.9	89.0	89.1
14	CH	74.6	74.6	74.4	75.4	74.7	74.4	74.1	73.8
15	CH_3	17.5	17.5	17.4	17.6	17.4	17.3	17.2	17.2
	CO	170.5	170.4	170.3		170.6	170.6	170.5	
	COCH3	21.0	21.0	20.9		21.2	21.3	21.1	
	OCH3	55.5				55.5			

[a]n.o.: not observed.

Table 2. ^1H NMR (400.0 MHz, $CDCl_3$) Data for Compounds 1−4 at 20 °C

pos	1 (J in Hz)	2 (J in Hz)	3 (J in Hz)	4 (J in Hz)
1	6.06, dd (5.7, 2.0)	6.06, dd (5.7, 1.8)	6.09, dd (5.7, 1.9)	6.06, dd (5.7, 1.9)
3	5.55, dd (5.7, 5.6)	5.53, dd (6.0, 5.7)	5.51, dd (5.8, 5,7)	5.55, dd (5.9, 5.7)
4	4.30, dd (8.9, 5.6, 2.0)	4.26, ddd (8.8, 6.0, 1.8)	4.24, ddd (9.0, 5.8, 1.9)	4.28, ddd (9.1, 5.9, 1.9)
5	6.10, br dd (15.9, 8.9)	6.16, br dd (16.0, 8.8)	6.24, br dd (15.7, 9.0)	6.08, dd (15.5, 9.1)
6	5.87, br dd (15.9, 6.5)	6.00, br dd (16.0, 6.4)	5.98, br dd (15.7, 7.2)	5.88, dd (15.5, 5.9)
7	3.66, br ddd (8.2, 6.5, 4.7)	4.13, br ddd (11.2, 6.4, 2.4)	4.27, br ddd (7.5, 7.2, 1.9)	4.20, m
8a	2.37, m	2.44, br ddd (12.6, 11.2, 10.9)	2.39, m	2.34, ddd (12.1, 8.2, 2.8)
8b	2.10, m	2.13, br ddd (12.6, 4.9, 2.4)	2.74, ddd (12.2, 11.1, 1.9)	2.10, ddd (12.1, 5.3, 3.9)
9	4.98, ddd (11.2, 4.7, 1.7)	4.99, ddd (10.9, 4.9, 1.9)	4.94, ddd (11.1, 5.2, 1.9)	3.76, ddd (8.2, 8.1, 3.9)
10	3.52, m	3.47, br ddd (11.9, 4.0, 1.9)	3.45, dd (11.9, 11.1)	3.43, dd (8.1, 7.9)
11a	2.40, m	2.39, br ddd (12.1, 7.7, 4.0)	2.37, dd (11.7, 7.8)	2.42, dd (8.2, 7.9)
11b	2.06, ddd (12.2, 11.9, 8.2)	2.06, ddd (12.1, 11.9, 8.3)	2.06, ddd (11.9, 11.7, 8.5)	2.42, dd (8.2, 7.9)
12	3.77, ddd (8.2, 7.9, 3.7)	3.75, ddd (8.3, 7.7, 3.9)	3.75, ddd (8.5, 7.8, 3.9)	3.78, m
13	4.04, dd (10.0, 3.7)	4.05, dd (9.8, 3.9)	4.05, dd (9.9, 3.9)	4.05, dd (9.7, 3.8)
14	3.16, dd (10.0, 6.3)	3.16, dd (9.8, 6.4)	3.12, dd (9.9, 6.4)	3.18, dd (9.7, 6.5)
15	1.23, d (6.3)	1.23, d (6.4)	1.22, d (6.4)	1.24, d (6.5)
$COCH_3$	2.14, s	2.15, s	2.15, s	
OMe	3.26, s			

(CH), δ_C 74.1 (CH), δ_H 6.06, δ_H 5.55], a *trans*-configured double bond [δ_H 6.10 (1H, br dd, J = 15.9, 8.9 Hz) and 5.87 (1H, br dd, J = 15.9, 6.5 Hz)], and an acetoxy group [δ_C 170.5 (C), δ_C 21.0 (CH_3), δ_H 2.14 (s)], along with two methylenes, and seven methines bonded with either a halogen, oxygen atom, or methyl (Tables 1 and 2). Eighteen ^{13}C NMR signals were expected; however only 14 were observed. Indeed, no direct ^1J coupling was observed in the HSQC spectrum for both olefinic protons [δ_H 6.10, 5.87]. However, additional information was obtained from the COSY and HMBC experiments: a methoxy displaying a chemical shift at 55.5 ppm was linked to the methine at 73.8 ppm, while the acetoxy group was linked to the methine at 68.3 ppm. The methine resonating at 44.9 ppm indicated a bromine substitution in agreement with the characteristic isotopic pattern for two bromine atoms observed in the mass spectrum. Detailed interpretation of the COSY and HMBC spectra led to the planar structure shown in Figure 1.

No long-range correlations through the ether linkage could be observed either in the COSY or the HMBC experiment. Broad signals observed in ^1H and ^{13}C NMR spectra at room temperature suggest the occurrence of an equilibrium between

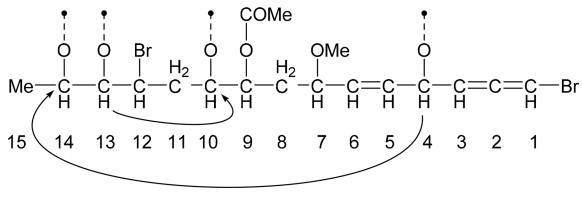

Figure 1. Planar structure of compound **1** and key HMBC correlations observed at −40 °C.

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different conformers (Figure 2). Among the C_{15} acetogenins, obtusallenes I to IV have been previously described as

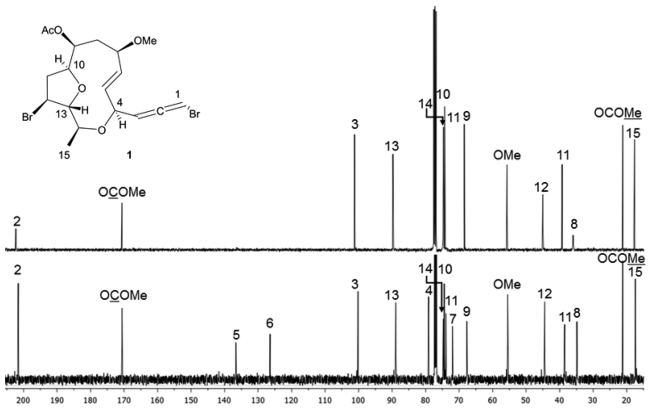


Figure 2. ^{13}C NMR spectrum of **1** at +20 °C (top) and −40 °C (bottom).

temperature-dependent conformers attributable to a major conformer in equilibrium with a minor one.[3] A full set of 1D and 2D NMR experiments were thus recorded at −40 °C (Supporting Information, Figures S2, S4, S5, S6, S8). Under these conditions the HMBC spectrum revealed long-range correlations from H-4 (δ_H 4.24) to C-14 (δ_C 74.7) and from H-13 (δ_H 3.98) to C-10 (δ_C 73.9), which established the two ether linkages between these positions (Figure 1). Compound **1** exhibits a 12-membered central acetogenic ring and a five-membered ring identical to obtusallene III.

The relative configuration of the stereogenic centers was determined based on H,H-coupling constant analysis and NOESY data (Figure S9). NOESY correlations from H-14 to H-4 and H-12 indicated that the methyl (C-15) together with the bromoallene unit and bromine at C-12 had a *syn* orientation (Figure 3). Correlations from H-10 to H-12 and from H-15 to

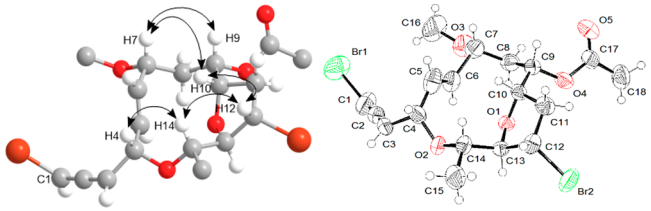


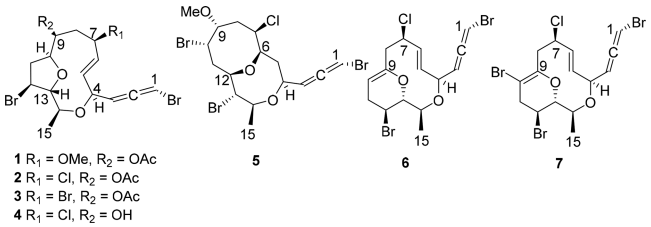
Figure 3. Key NOESY correlations and crystal structure of **1** (ORTEP drawing, 50% probability level ellipsoids).

H-13 revealed that the tetrahydrofuran ring closure was *trans*. The configuration of C-9 was established on the basis of the NOESY cross-peak observed between H-9 and H-10 and the small coupling constant of $^3J_{H\text{-}9,H\text{-}10}$ = 1.7 Hz. NOESY enhancement observed from H-7 to H-10 and H-9 indicated that the methoxy and the acetoxy groups also had a *syn* orientation.

In conclusion, NMR analysis indicated that all substituents of the 12-membered central acetogenic ring including the bromoallene unit are located on the same side of the molecule, while electronic circular dichroism (ECD) analysis indicted an *R* configuration for the bromoallene unit.[13,14] However, the relationship between these two chiral subunits and thus the absolute configuration of the molecule could not be deduced but ultimately required X-ray crystallographic analysis. Suitable quality crystals of compound **1** were obtained from recrystallization in $CHCl_3$, the analysis of which confirmed the relative configuration deduced from the NMR data and established structural features and the absolute configuration of **1** as a*R*,4*S*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S* (Figure 3).

C_{15} acetogenins **2**, **3**, and **4** were obtained as optically active white powders, and their molecular formulas were established by HRESIMS to be $C_{17}H_{21}Br_2ClO_4$, $C_{17}H_{21}Br_3O_4$, and $C_{15}H_{19}Br_2ClO_3$, respectively. The ^1H NMR spectra of **2**−**4** showed strong similarities with **1** (Table 2). However, the characteristic ^1H NMR signal for the methoxy group was missing. Based on the ^{13}C NMR chemical shifts observed at −40 °C for C-7 (71.9 (**1**) vs 66.6 (**4**) vs 64.1 (**2**) vs 50.5 (**3**)) and the molecular formulas, **2** and **4** possess a chlorine atom at C-7 and **3** contains a bromine atom at C-7 (Table 1). Moreover, the characteristic ^1H and ^{13}C NMR signals of the acetoxy group were missing in the spectra of **4**. Based on the ^{13}C NMR chemical shifts and the molecular formula, **4** carries a free hydroxy group at position C-9. Similar to **1**, missing and broad ^{13}C and ^1H NMR signals were observed at +20 °C for compounds **2**−**4** due to equilibrating conformers. Therefore, a full set of 1D and 2D NMR spectra were recorded at −40 °C to obtain data for the major conformers. However, the coalescence temperature was not reached for compound **4**, even at −50 °C. The relative configurations of compounds **2**−**4** were deduced by a detailed interpretation of ^1H NMR coupling constants and NOE correlations. Compounds **1**−**4** displayed identical relative configurations for all of the stereogenic centers. According to experimental ECD spectra, specific rotation values, and the assumption of a common biosynthesis, it seems reasonable to assume that compounds **2**−**4** also share the same absolute configuration as **1** and are (a*R*,4*S*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-12-bromo-7-chloro-obtusallenyl acetate III (**2**), (a*R*,4*S*,7*R*,9*S*,10*S*,12*S*,13*R*,14*S*)-7,12-dibromo-obtusallenyl acetate III (**3**), and (a*R*,4*S*,7*R*,9*S*,10*S*,12*S*,-13*R*,14*S*)-12-bromo-7-chloro-obtusallene III (**4**) (Chart 1).

Chart 1



The C_{15} acetogenin **5** was obtained as an optically active white powder, and its molecular formula was established by HRESIMS to be $C_{16}H_{24}Br_2ClO_4$, corresponding to four degrees of unsaturation. Interrogation of our ^{13}C NMR library initially suggested the molecule to be the previously described marilzabicycloallene C.[7] However, only 13 of the 16 carbons possessed highly similar chemical shift values ($\Delta\delta \leq 0.5$ ppm). The dominating chemical shift variation was observed for carbon C-12 (74.7 vs 57.0). This strongly shielded signal is due to a bromine substitution at C-12. The molecular formula and the γ-steric effects observed for the methyl group at C-15 ($\Delta\delta$ = 3.2 ppm) and the methylene group C-11 ($\Delta\delta$ = 1.9 ppm) are consistent with the presence of a bromine at C-12. Furthermore, the ^1H and ^{13}C NMR spectra of **5** exhibited resonances for a bromoallene unit [δ_C 200.9 (C), δ_C 102.7

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(CH), δ_C 74.2 (CH), δ_H 6.07, δ_H 5.44], three methylene groups, and eight methine units bonded with either a halogen or oxygen atom or one methyl group (Table 3). Detailed

Table 3. ^{13}C NMR (125.5 MHz) and ^{1}H NMR (400.0 MHz, $CDCl_3$) Data for Compound 5

pos	δ_C, type	δ_H (J in Hz)
1	74.2, CH	6.07, dd (5.7, 2.1)
2	200.9, C	
3	102.7, CH	5.44, dd (5.7, 5.5)
4	81.2, CH	4.37, dddd (11.1, 5.5, 2.1, 1.5)
5	38.4, CH_2	2.18, ddd (14.6, 11.1, 10.9)
		1.48, ddd (14.6, 1.6, 1.5)
6	86.6, CH	3.94, ddd (10.9, 1.6, 1.5)
7	63.7, CH	4.17, ddd (5.5, 1.8, 1.5)
8	40.3, CH_2	2.88, ddd (16.2, 7.2, 1.8)
		2.16, ddd (16.2, 5.5, 1.0)
9	78.9, CH	4.0, ddd (7.2, 1.0, 0.9)
10	60.7, CH	4.81, ddd (4.3, 3.3, 0.9)
11	44.1, CH_2	2.95, ddd (15.1, 4.3, 1.0)
		2.30, ddd (15.1, 10.3, 3.3)
12	84.8, CH	4.05, ddd (10.3, 10.0, 1.0)
13	57.0, CH	3.65, dd (10.0, 9.8)
14	85.0, CH	3.84, dd (9.8, 6.1)
15	23.8, CH_3	1.54, d (6.1)
16	56.1 (OCH_3)	3.37, s

interpretation of COSY, HMBC, and HRMS data confirmed the presence of a [5.5.1]bicylotridecane ring system characteristic for marilzabicycloallene. The relative configuration was finally established based on the $^{3}J_{H,H}$ coupling constants and interpretation of the NOESY spectrum. Compound **5** has the relative configuration 4*S**,6*R**,7*R**,9*R**,10*S**,12*R**,13*S**,14*S**, as well as displaying the bromoallene unit with an *R* configuration according to a negative Cotton effect and a negative specific rotation.[13,14]

In addition to the new metabolites **1**−**5**, 17 known natural products were identified by comparison of ^{13}C NMR spectroscopic data with those compiled in our in-house library. The direct analyses of the extract by ^{13}C NMR led to the identification of six known compounds, among which four were C_{15} acetogenins, 3-(*E*)-laurenyne,[15] 3-(*Z*)-laurenyne,[16] obtusallene I (**6**), and 10-bromo-obtusallene I (**7**);[3] one sesquiterpene, 9,15-dibromo-1,3(15)-chamigradien-11-ol;[17] and one sterol, cholesterol.[18] 3-(*E*)-Laurenyne represents by far the most abundant compound from the EtOAc extract. Detailed investigation of chromatographic fractions allowed the identification of four additional acetogenins, 4-acetoxymarilzallene,[9] marilzallene B,[19] obtusin,[20] and (*E*)-pinnatifidenyne,[21] and seven sesquiterpenes, laurene,[21] dihydrolaurene,[22] α-bromocuparene,[23] α-isobromocuparene,[23] cycloelatenene A,[24] α-snyderol,[25] and 1-deacetoxy-8-deoxyalgoave (Figure S59).[26]

Proposed Biosynthesis of Compounds 1−4. The structures of the new obtusallenes **1**−**4** are similar to those of obtusallene III, for which a biogenetic pathway has been proposed by Braddock.[4] However, **1**−**4** differ by the substituents at positions C-7 and C-9, as well as the substituent and relative configuration at position C-12 (Figure 4). Interestingly, the occurrence of bromine at position C-7 in compound **3** has not yet been reported in any obtusallene. Indeed, obtusallenes V, VI, and VII previously described as brominated at C-7 have subsequently been reassigned to carry the bromine at position C-12.[27,28] As shown in Figure 4, compound **3** can be generated by attack of bromide ion at position C-7 in an oxonium intermediate, which might also serve as a precursor to compound **1**. However, we cannot exclude that compound **3** originates from a bromide-mediated opening of the epoxide ring in the (*Z*)-6,7-epoxide derived from *Z*,*Z*,*Z*-hexadeca-4,7,10-trienoic acid.

In conclusion, we found that specimens of *Laurenciella* sp. from the Mediterranean Sea produce obtusallene III analogues and a marilzabicycloallene derivative, whereas specimens from the Atlantic Ocean produce obtusallene IV derivatives and marilzabicycloallenes.[7−9] Moreover, only 4-acetoxymarilzallenes were identified in both *Laurenciella* from the Canary and Corsica Islands. The occurrence of closely related compounds together with the convergence in the large chain gene of the ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) pro-

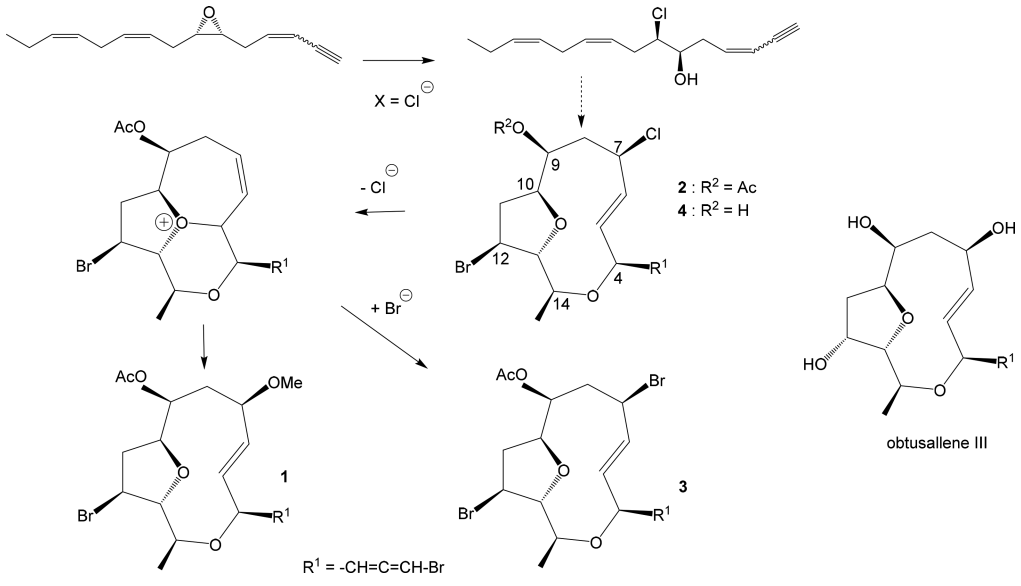

Figure 4. Hypothetical biosynthetic pathway to **1**−**4**.

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vides strong evidence that this Mediterranean Sea species belongs to the genus *Laurenciella*. Further genomic and metabolomic investigations will need to be conducted to establish if Atlantic and Mediterranean specimens are conspecific or represent distinct species.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points was measured using a STUART SMP30 melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-2000 polarimeter. IR spectra was recorded on a ThermoFisher Scientific Nicolet iS5 FTIR spectrometer. UV and ECD spectra were recorded on a JASCO J-1500 spectrometer. Room-temperature NMR spectra were recorded on a Bruker AVANCE 400 Fourier transform spectrometer, equipped with a 5 mm BBOF probe, in $CDCl_3$, with all shifts referenced to internal tetramethylsilane (TMS). ^{13}C NMR spectra of extracts and chromatographic fractions were recorded with the following parameters: pulse width = 4 μs (flip angle 45°); acquisition time = 2.7 s for 128 K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3000 for most mixtures (when available, around 40 mg of the sample in 0.5 mL of $CDCl_3$). ^1H, ^{13}C, and 2D NMR spectra of pure compounds were recorded using Bruker pulse programs. ^1H NMR and ^{13}C NMR spectra at variable temperature were recorded in $CDCl_3$, on a Bruker AV 400 spectrometer with a BVT3000 variable-temperature unit. HRMS spectra were recorded by direct infusion of samples dissolved in MeOH (1)/H_2O (1)/MeCN (1) in both positive and negative ESI mode using a Waters Synapt G2 QTOF mass spectrometer.

Biological Material. Specimens of *Laurenciella* sp. (Rhodomelaceae) were collected in the Mediterranean Sea in June 2014 at 20 to 100 cm depth, along the Sanguinaires Road, Ajaccio, Corsica, France (41°54′25″ N, 8°39′36″ E). A voucher specimen (H8316) has been deposited in the Centre d'Océanologie de Marseille, University of Aix-Marseille II. Initially it was identified as a species of *Laurencia* in the field. However, DNA sequences of the genes coding for the mitochondrial cytochrome oxidase 1 and the plastidal RuBisCO large subunit were used to assess the relationships of this species (sequences and specimen information available in BOLD under the process ID: LALA001-17).[29] A molecular systematics approach using distance and ML methods both revealed that the specimen allied with the recently described genus *Laurenciella*.[30] Moreover, the specimen differed from the only species so far included in this genus by 0.5% divergence in their rbcL and 4.95% in their CO1. This level of divergence is usually related to inter specific divergence;[31] we therefore concluded that our specimens belong to a nondetermined species of *Laurenciella*. In the absence of reproductive features, we refrain from describing a novel species.

Extraction and Isolation. The specimens of *Laurenciella* sp. were cleaned from epiphytes, freeze-dried, and frozen under liquid N_2, ground using a mortar, and then extracted with EtOAc at room temperature to obtain a dark green, viscous oil (2.9 g). The extract was first chromatographed using a Grace Reveleris flash chromatography system equipped with a Reveleris flash Cartridge (silica gel 40 g, 40 μm) in dry-load mode using a gradient of pentane/$CHCl_3$, $CHCl_3$/EtOAc, and finally EtOAc/MeOH. Seventy-four fractions (F) were collected. F16 (4.2 mg, 20% $CHCl_3$/80% pentane) contains **6**. F18–21 (16.1 mg, 20% $CHCl_3$/80% pentane) were combined and submitted to series of column chromatography (silica gel, 63–200 μm, 15 g) to afford **5** (2.1 mg). F32–34 (55.3 mg, 25% $CHCl_3$/75% pentane) were combined and submitted to a series of column chromatography (silica gel, 63–200 μm, 25 g, and Sephadex LH-20, 30 g in MeOH) to afford **3** (8.9 mg). F36–39 (73.3 mg, 50% $CHCl_3$/50% pentane) were combined and submitted to a series of column chromatography (silica gel, 63–200 μm, 35 g, and Sephadex LH-20, 40 g in MeOH) to afford **1** (39.9 mg). F46 and F47 (22.2 mg, 75% $CHCl_3$/25% pentane) were combined and submitted to a series of column chromatography (silica gel, 63–200 μm, 23 g, and (Sephadex LH-20, 24 g in MeOH) to afford **2** (8.9 mg). F51 and F52 (18.4 mg, 80% $CHCl_3$/20% pentane) were combined and submitted to a series of column chromatography (silica gel, 63–200 μm, 20 g, and Sephadex LH-20, 21 g in MeOH) to afford **4** (4.2 mg).

*(−)-(aS,4R,7S,9R,10R,12R,13S,14R)-12-Bromo-7-methoxy-obtusallenyl acetate III (**1**):* colorless needeles; mp 133–135 °C; $[\alpha]^{25}_D$ −45 (*c* 2.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 200 nm (0.82); ECD (*c* 0.5 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 250 (−25.8), 275 nm (−48.7); IR (ATR) ν_{max} 2921, 2900, 2836, 1921, 1742, 1690, 1389, 1252, 1158, 1105, 1060, 953, 774, 700, 655 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, −40 °C) δ 6.09 (1H, dd, *J* = 16.0, 9.5 Hz, H-5), 6.03 (1H, dd, *J* = 5.6, 1.6 Hz, H-1), 5.82 (1H, dd, *J* = 16.0, 6.1 Hz, H-6), 5.52 (1H, dd, *J* = 5.6, 5.5 Hz, H-3), 4.90 (1H, br dd, *J* = 11.2, 4.3 Hz, H-9), 4.24 (1H, ddd, *J* = 9.5, 5.5, 1.6 Hz, H-4), 3.98 (1H, dd, *J* = 9.9, 3.7 Hz, H-13), 3.71 (1H, ddd, *J* = 8.4, 7.6, 3.7 Hz, H-12), 3.67 (1H, dd, *J* = 6.1, 5.5, 1.9 Hz, H-7), 3.41 (1H, br dd, *J* = 11.2, 9.6 Hz, H-10), 3.22 (3H, s, OMe), 3.12 (1H, dd, *J* = 9.9, 6.2 Hz, H-14), 2.35 (1H, ddd, *J* = 11.7, 7.6, 3.5 Hz, H-11β), 2.31 (1H, dd, *J* = 11.9, 11.7 Hz, H-8β), 2.12 (3H, s, OCOC$\underline{H}_3$), 2.06 (1H, m, H-8α), 2.00 (1H, dd, *J* = 11.7, 9.6 Hz, H-11α), 1.18 (3H, d, *J* = 6.2 Hz, H-15); HRESIMS *m*/*z* 500.9897, 502.9876, 504.9860 $[M + Na]^+$ (52:100:53) (calcd for $C_{18}H_{24}{}^{79}Br_2O_5Na$, 500.9889; $C_{18}H_{24}{}^{79}Br^{81}BrO_5Na$, 502.9868; $C_{18}H_{24}{}^{81}Br_2O_5Na$, 504.9848).

*(−)-(aS,4R,7S,9R,10R,12R,13S,14R)-12-Bromo-7-chloro-obtusallenyl acetate III (**2**):* colorless, amorphous solid; $[\alpha]^{25}_D$ −63 (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 200 nm (0.62); ECD (*c* 0.5 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−16.8), 275 nm (−2.4); ^1H NMR ($CDCl_3$, 400 MHz, −40 °C) δ 6.19 (1H, dd, *J* = 15.5, 9.3 Hz, H-5), 6.09 (1H, d, *J* = 5.3 Hz, H-1), 6.00 (1H, dd, *J* = 15.5, 6.4 Hz, H-6), 5.53 (1H, dd, *J* = 5.9, 5.3 Hz, H-3), 4.95 (1H, br dd, *J* = 10.5, 3.8 Hz, H-9), 4.27 (1H, br dd, *J* = 9.3, 5.9 Hz H-4), 4.11 (1H, m, H-7), 4.03 (1H, dd, *J* = 9.9, 3.6 Hz, H-13), 3.73 (1H, ddd, *J* = 11.5, 8.4, 3.6 Hz, H-12), 3.40 (1H, br d, *J* = 10.5 Hz, H-10), 3.15 (1H, br dd, *J* = 9.9, 6.1 Hz, H-14), 2.43 (1H, m, H-8β), 2.40 (1H, m, H-11β), 2.18 (3H, s, $OCOCH_3$), 2.12 (1H, m, H-8α), 2.04 (1H, ddd, *J* = 11.8, 11.5, 10.5 Hz, H-11α), 1.22 (3H, d, *J* = 6.1 Hz, H-15); HRESIMS *m*/*z* 498.9535, 500.9517, 502.9498, 504.9488 $[M + H_2O - H]^-$ (48:100:76:15) (calcd for $C_{17}H_{22}{}^{79}Br_2{}^{35}ClO_5$, 498.9523; $C_{17}H_{22}{}^{79}Br^{81}Br^{35}ClO_5$, 500.9502; $C_{17}H_{22}{}^{81}Br_2{}^{35}ClO_5$, 502.9482, $C_{17}H_{22}{}^{81}Br_2{}^{37}ClO_5$, 504.9452); *m*/*z* 486.9736, 488.9714, 490.9702 $[M - HCl + H_2O + Na]^+$ (51:100:52) (calcd for $C_{17}H_{22}{}^{79}Br_2O_5Na$, 486.9732; $C_{17}H_{22}{}^{79}Br^{81}BrO_5Na$, 488.9712; $C_{17}H_{22}{}^{81}Br_2O_5Na$, 490.9691).

*(−)-(aS,4R,7S,9R,10R,12R,13S,14R)-7,12-Dibromo-obtusallenyl acetate III (**3**):* colorless, amorphous solid; $[\alpha]^{25}_D$ −37 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 200 nm (0.82); ECD (*c* 0.5 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−10.7), 275 nm (−2.2); ^1H NMR ($CDCl_3$, 400 MHz, −40 °C) δ 6.25 (1H, dd, *J* = 15.7, 9.7 Hz, H-5), 6.09 (1H, dd, *J* = 5.5, 1.2 Hz, H-1), 5.94 (1H, dd, *J* = 15.7, 6.8 Hz, H-6), 5.51 (1H, dd, *J* = 6.0, 5.5 Hz, H-3), 4.89 (1H, dd, *J* = 10.6, 4.7 Hz, H-9), 4.26 (1H, m, H-7), 4.24 (1H, m, H-4), 4.01 (1H, dd, *J* = 9.5, 3.6 Hz, H-13), 3.72 (1H, ddd, *J* = 11.7, 8.2, 3.6 Hz, H-12), 3.38 (1H, br d, *J* = 11.4 Hz, H-10), 3.10 (1H, dd, *J* = 9.5, 6.1 Hz, H-14), 2.70 (1H, m, *J* = 10.6, 6.0 Hz, H-8β), 2.38 (1H, m, H-11β), 2.36 (1H, m, H-8α), 2.16 (3H, s, $OCOCH_3$), 2.04 (1H, ddd, *J* = 11.7, 11.2, 10.6 Hz, H-11α), 1.20 (3H, d, *J* = 6.1 Hz, H-15); HRESIMS *m*/*z* 556.9185, 558.9171, 560.9152, 562.9134 $[M + CH_3OH - H]^-$ (34:100:100:0.35) (calcd for $C_{18}H_{24}{}^{79}Br_3O_5$, 556.9174; $C_{18}H_{24}{}^{79}Br_2{}^{81}BrO_5$, 558.9154; $C_{18}H_{24}{}^{79}Br\ {}^{81}Br_2O_5$, 560.9133; $C_{18}H_{24}{}^{81}Br_3O_5$, 562.9113) *m*/*z* 500.9895, 502.9873, 504.9855 $[M - HBr + CH_3OH + Na]^+$ (53:100:51) (calcd for $C_{18}H_{24}{}^{79}Br_2O_5Na$, 500.9888; $C_{18}H_{24}{}^{79}Br^{81}BrO_5Na$, 502.9868; $C_{18}H_{24}{}^{81}Br_2O_5Na$, 504.9848).

*(−)-(aS,4R,7S,9R,10R,12R,13S,14R)-12-Bromo-7-chloro-obtusallene III (**4**):* colorless, amorphous solid; $[\alpha]^{25}_D$ −58 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 200 nm (0.35); ECD (*c* 0.25 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (−3.2), 275 nm (−1.3); HRESIMS *m*/*z* 456.9429, 458.9405, 460.9385, 462.9368 $[M + H_2O - H]^-$ (48:100:77:14) (calcd for $C_{15}H_{20}{}^{79}Br_2{}^{35}ClO_4$, 456.9417; $C_{15}H_{20}{}^{79}Br^{81}Br^{35}ClO_4$, 458.9397; $C_{15}H_{20}{}^{81}Br_2{}^{35}ClO_4$, 460.9376, $C_{15}H_{20}{}^{81}Br_2{}^{37}ClO_4$, 462.9347); *m*/*z* 444.9630, 446.9606, 448.9589 $[M - HCl + H_2O + Na]^+$ (49:100:51) (calcd for $C_{15}H_{20}{}^{79}Br_2O_4Na$, 444.9626; $C_{15}H_{20}{}^{79}Br^{81}BrO_4Na$, 446.9606; $C_{15}H_{20}{}^{81}Br_2O_4Na$, 448.9585).

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*12-Bromo-9-methoxymarilzabicycloallene C (**5**):* colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 200 nm (0.89); $[\alpha]^{25}_{D}$ −71 (*c* 0.1, $CHCl_3$); ECD (*c* 0.25 mg/mL, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (−11.2), 275 nm (−1.2); HRESIMS *m*/*z* 520.8943, 522.8922, 524.8901, 526.8875 $[M - HCl + Na]^+$ (36:100:100:35) (calcd for $C_{16}H_{21}{}^{79}Br_3O_3Na$, 520.8939; $C_{16}H_{23}{}^{79}Br_2{}^{81}BrO_3Na$, 522.8918; $C_{16}H_{23}{}^{79}Br\ {}^{81}Br_2O_3Na$, 524.8898; $C_{16}H_{23}\ {}^{81}Br_3O_3Na$, 526.8877).

*Crystal Data for **1**:* $C_{18}H_{24}Br_2O_5$, M = 480.19, 0.43 × 0.18 × 0.16 mm^3, monoclinic, space group $P2_1$, a = 4.7521(3) Å, b = 25.2432(16) Å, c = 17.1496(11) Å, V = 2057.1(2) $Å^3$, Z = 4, D = 1.550 mg/m^3, μ = 3.964 mm^{-1}, T = 293 K, 11 216 refractions collected, 5616 [R(int) = 0.0618] independent reflections, final R_1 = 0.0656 (wR_2 = 0.1563). Flack parameter = −0.014(7). Colorless crystals of **1** were obtained from recrystallization in $CHCl_3$ and were mounted on a Stoe Mark II image plate diffraction system, using Mo Kα graphite-monochromated radiation, image plate distance 135 mm, 2θ range from 3.2° to 58.7°, D_{max} − D_{min} = 16.029−0.836 Å. The structure was solved by direct methods using the program SHELXS-97.[32] Refinement and all further calculations were carried out using SHELXL-97. The H atoms were included in calculated positions and treated as riding atoms using the SHELXL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2, except for the minor components of the disordered olefinic group, which has remained isotropic. In one of the two independent molecules, the olefinic group was disordered over two positions, and the occupation factors were refined to 53:47 using a free variable. The assignment of the absolute configuration of **1** was based on the Flack parameter (−0.014(7)), which was further confirmed by generating the opposite configuration, for which a Flack parameter of 1.015(7) was obtained. Crystallographic details of compound **1** are summarized in Supporting Information Table S60. Figure 3 was drawn with ORTEP.[33]

Crystallographic data (excluding structure factor tables) have been deposited in the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-1589315. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB1EZ, UK (fax: int. + (1223) 336 033); e-mail: deposit@ccdc.cam.ac.uk).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00706.

Picture of the specimen of the population investigated; 1D and 2D NMR spectra at +20 and −40 °C; HRMS spectra of compound **1**−**5**; UV and ECD spectra of compounds **1**−**5**; structures of known compounds identified in this study (PDF)

X-ray crystallographic data for **1** (CIF)

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Notes

The authors declare no competing financial interest.

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