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New Bicyclotridecane C_{15} Nonterpenoid Bromoallenes from *Laurencia marilzae*

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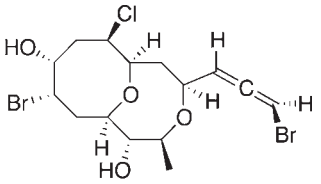
ABSTRACT

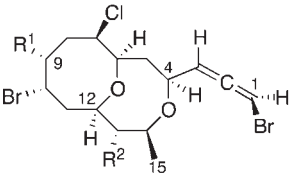


Marilzabicycloallene A (1)

Nonterpenoid bromoallenes possessing a novel skeleton that incorporates an unprecedented [5.5.1]bicyclotridecane ring system, marilzabicycloallenes A–D (1–4), were isolated from specimens of *Laurencia marilzae* collected on the Canary Islands. The framework of these metabolites strongly reinforces Braddock's hypothesis concerning the biosynthesis via electrophilic bromination of the obtusallene family.

The Laurencia genus produces a host of halogenated nonterpenoid C_{15} compounds, which have generally been accepted as arising from a fatty acid metabolism.[1] Members of this family include unusual cyclic ethers, typically with a 5- to 9-membered central acetogenic oxygen, an enyne or allene unit, and at least one bromine atom.[2] An interesting subset of these halogenated C_{15} acetogenins is the obtusallenes, which incorporate a 12-membered *O*-heterocycle in their structures.[3]

In 2006, Braddock proposed a hypothesis for the biosynthesis of the obtusallene family involving multiple electrophilic bromination.[4] The hypothesis correctly predicted the stereochemistries of obtusallenes I–IV, whose structures have been unambiguously solved by X-ray crystallography, but questioned the structures of obtusallenes V–VII solved by NMR spectroscopy, which show a bromine atom at C-7 and a chlorine atom at C-13.[5,6] Subsequently, to validate the proposed biogenesis and resolve the controversy about the reported structures of obtusallenes V–VII, Braddock et al. experimentally proved the viability of the bromonium ion induced transannular oxonium ion formation–fragmentation to give macrocyclic carbon frameworks of obtusallene VII with a bromine atom at C-13 in line with their published hypothesis.[7] Interestingly, a brominated [5.5.1]bicyclotridecane adduct was also isolated in the study and the authors speculated that it may represent the core of an, as yet, undiscovered natural product from the *Laurencia* species. As part of our ongoing study of a new species of Laurencia, four new compounds presenting this unique framework in the field of marine natural products were obtained from *Laurencia marilzae*.[8,9] Herein, we report the isolation and structure elucidation of marilzabicycloallenes A–D (**1**–**4**), as well as consider their biogenetic origin.



1 $R^1 = R^2 = OH$
3 $R^1 = OCH_3$, $R^2 = OH$
4 $R^1 = R^2 = Cl$

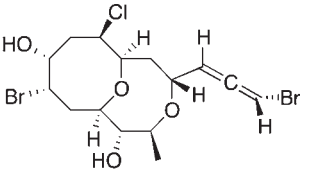


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Table 1. ^{1}H NMR Data of Marilzabicycloallenes A-D (**1**-**4**)[a]

	marilzabicycloallene A (**1**)	marilzabicycloallene B (**2**)	marilzabicycloallene C (**3**)	marilzabicycloallene D (**4**)
position	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)
1	6.05, dd (2.1, 5.7)	6.03, dd (1.6, 5.7)	6.05, dd (2.1, 5.6)	6.07, dd (2.1, 5.6)
3	5.47, dd (5.5, 5.7)	5.42, dd (5.7, 6.5)	5.47, dd (5.5, 5.6)	5.43, dd (5.5, 5.6)
4	4.33, dddd (1.8, 2.1, 5.5, 11.3)	4.34, dddd (1.6, 4.7, 6.5, 10.9)	4.33, dddd (2.0, 2.1, 5.5, 11.3)	4.35, dddd (2.1, 2.1, 5.5, 11.2)
5	β 2.13, ddd (10.9, 11.3, 14.5)	β 2.09, ddd (4.7, 10.8, 13.7)	β 2.13, ddd (10.9, 11.3, 14.8)	β 2.18, ddd (11.2, 11.2, 14.7)
	α 1.48, ddd (1.8, 1.8, 14.5)	α 1.68, ddd (1.8, 10.9, 13.7)	α 1.49, ddd (1.7, 2.0, 14.8)	α 1.52, ddd (1.7, 2.1, 14.7)
6	3.87, ddd (1.8, 1.8, 10.9)	3.98, ddd (1.8, 1.9, 10.8)	3.89, ddd (1.7, 1.9, 10.9)	3.93, ddd (1.7, 1.9, 11.2)
7	4.14, ddd (1.8, 1.9, 5.7)	4.15, ddd (1.8, 1.9, 5.6)	4.18, ddd (1.8, 1.9, 5.7)	4.06, ddd (1.9, 1.9, 5.4)
8	α 2.98, ddd (1.9, 7.4, 15.8)	α 2.99, ddd (1.8, 7.5, 15.7)	α 2.90, ddd (1.8, 7.1, 16.0)	α 3.24, ddd (1.9, 7.8, 16.4)
	β 2.11, ddd (1.1, 5.7, 15.8)	β 2.16, dddd (0.8, 0.8, 5.6, 15.7)	β 2.17, ddd (1.1, 5.7, 16.0)	β 2.62, dddd (1.1, 1.1, 5.4, 16.4)
9	4.58, ddd (1.1, 1.3, 7.4)	4.54, br d (7.5)	4.06, ddd (1.0, 1.1, 7.1)	5.01, ddd (1.1, 1.1, 7.8)
10	4.83, ddd (1.3, 3.5, 4.0)	4.86, br dd (3.5, 4.2)	4.85, ddd (1.0, 3.3, 4.1)	4.96, ddd (1.1, 3.2, 4.2)
11	α 2.59, ddd (1.3, 4.0, 15.3)	α 2.53, ddd (1.3, 4.2, 15.2)	α 2.59, ddd (1.4, 4.1, 15.1)	α 2.78, ddd (1.2, 4.2, 15.1)
	β 2.31, ddd (3.5, 10.6, 15.3)	β 2.26, ddd (3.5, 11.2, 15.2)	β 2.27, ddd (3.3, 10.0, 15.1)	β 2.35, ddd (3.2, 10.5, 15.1)
12	3.71, ddd (1.3, 9.0, 10.6)	3.78, ddd (1.3, 8.8, 11.2)	3.74, ddd (1.4, 9.9, 10.0)	3.96, ddd (1.2, 9.8, 10.5)
13	3.26, dd (8.7, 9.0)	3.48, m	3.26, dd (9.0, 9.9)	3.51, dd (9.8, 9.8)
14	3.57, dd (6.2, 8.7)	3.68, dd (6.6, 8.8)	3.57, dd (6.2, 9.0)	3.75, dd (6.2, 9.8)
15	1.35, d (6.2)	1.42, d (6.6)	1.35, d (6.2)	1.46, d (6.2)
OCH_3			3.37, s	

[a] Data recorded at 600 MHz in $CDCl_3$ at 298 K

Table 2. ^{13}C NMR Data for Marilzabicycloallenes A-D (**1**-**4**)[a]

		1	**2**	**3**	**4**
position	mult.	δ_C	δ_C	δ_C	δ_C
1	CH	74.1	73.9	74.1	74.3
2	C	200.9	201.0	200.9	201.0
3	CH	103.2	102.8	103.2	102.7
4	CH	81.1	67.4	81.1	81.1
5	CH_2	38.7	37.8	38.7	38.3
6	CH	86.4	82.5	86.7	86.2
7	CH	63.6	63.1	63.9	63.4
8	CH_2	44.4	45	40.4	45.2
9	CH	70.3	70.4	79.0	60.1
10	CH	64.8	64.9	61.0	62.9
11	CH_2	41.7	40.7	42.2	44.1
12	CH	84.2	82.2	84.5	84.3
13	CH	74.7	76.0	74.7	63.0
14	CH	85.1	79.1	85.1	85.1
15	CH_3	20.6	18.1	20.6	22.4
OCH_3	CH_3			56.1	

[a] Data recorded at 150 MHz in $CDCl_3$ at 298 K

Marilzabicycloallene A (**1**) was isolated as an optically active white amorphous solid. The molecular formula was established as $C_{15}H_{21}Br_2ClO_4$ based on analysis of high-resolution electrospray ionization mass spectrometry data (m/z 480.9358, 482.9375, and 484.9342 [M + Na]$^+$) and the presence of 15 signals in its ^{13}C NMR spectrum. The IR spectrum showed bands attributed to hydroxy, allene and ether functions (3365, 1958, and 1074 cm^{-1}). Signals observed in its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) agree with the presence of eight heteroatom-bearing methines (six methines flanked by oxygen and two by halogen atoms), three methylenes, and one secondary methyl group. Also the characteristic resonances for a bromoallene functionality (δ_C 200.9 s, 103.2 d, 74.1 d; δ_H 6.05 dd, J = 2.1, 5.7 Hz and 5.47 dd, J = 5.5, 5.7 Hz) were observed. Analysis of the COSY, HSQC and HMBC NMR spectra revealed the presence of only one large spin system in the structure comprising C-3→C-15 and with heteroatoms located on carbons C-4, C-6, C-7, C-9, C-10, C-12, C-13 and C-14 (Figure 1).

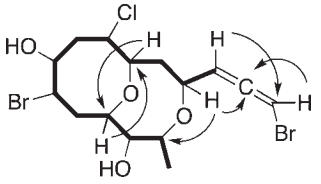

Figure 1. COSY (—) and selected HMBC (→) correlations of marilzabicycloallene A (**1**).

HMBC correlations observed from H-4 (δ_H 4.33) to C-14 (δ_C 85.1) and from H-6 (δ_H 3.87) to C-12 (δ_C 84.2), established two ether linkages between these positions, and therefore placed the remaining two hydroxy groups at C-9 and C-13. The relative configuration of **1** was determined on the basis of coupling constant analysis and ROESY

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data (Figure 2). ROESY enhancements observed from H-4 to H-14, H-6 and H-12, from H-6 to H-7 and one of the diasterotopic H-8 methylene proton resonances (H-8α) revealed an identical orientation for all of them, while the heterocycle ring closures were determined as *cis*. In addition, the large coupling constants between H-8α/H-9 ($^3J_{\text{H-8α,H-9}}$ = 7.4 Hz), H-12/H-13 ($^3J_{\text{H-12,H-13}}$ = 9.0 Hz), and H-13/H-14 ($^3J_{\text{H-13,H-14}}$ = 8.7 Hz) suggested an *anti* arrangement between them and located H-9 and H-13 on the opposite face of the molecule relative to H-8α, H-12 and H-14. The stereochemistry of C-10 was established on the basis of the ROESY cross-peak observed between H-9 and H-10 and the small coupling constant of $^3J_{\text{H-9,H-10}}$ = 1.3 Hz. The relative configuration of **1** was therefore determined to be 4*S**, 6*R**, 7*R**, 9*R**, 10*S**, 12*R**, 13*S** and 14*S**, as well as displaying the bromoallene unit with an *R* configuration.[10−12]

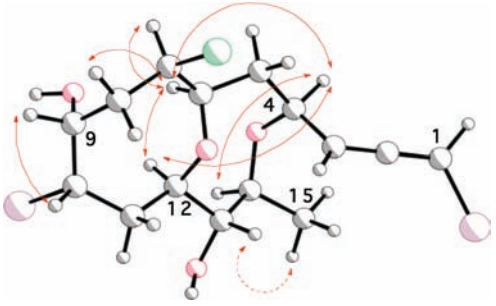

Figure 2. Key ROESY correlations of compound **1**.

Marilzabicycloallene B (**2**) was isolated as an optically active amorphous solid that gave three pseudomolecular $[M + Na]^+$ ions at *m*/*z* 480.9378, 482.9382, and 484.9413 in the ESI-HRMS spectrum consistent with the molecular formula $C_{15}H_{21}Br_2ClO_4$, identical to the molecular formula of marilzabicycloallene A (**1**). IR, as well as extensive assessment of 1D and 2D NMR data (Tables 1 and 2, see Supporting Information), indicated that compound **2** is closely related to marilzabicycloallene A (**1**). Marilzabicycloallene B (**2**) differs from **1** mainly in the chemical shifts of C-14 (δ_C 85.1 in **1** vs δ_C 79.1 in **2**) and C-4 (δ_C 81.1 in **1** vs δ_C 67.4 in **2**), evoking a different relative stereochemistry for C-4, similar to those described for other macrocyclic obtusallenes.[12,13] This contention was confirmed by a ROESY cross-peak observed between H-4 and H_3-15 and the absence of correlations between H-4 and H-14 or H-6. Additional ROESY enhancements from H-14 to H-6 and H-12 revealed the spatial proximity of these protons and thus determined H-4 to be on the β face of the molecule and an *R** configuration for this chiral center.

In addition, the existence of a positive Cotton effect for **2**, opposite to that of **1**, indicated a different configuration of the allene residue, which could be predicted as *S*.[10]

Marilzabicycloallene C (**3**) was determined to have the molecular formula $C_{16}H_{23}Br_2ClO_4$ from analysis of the NMR spectral data and ESI-HRMS ions at *m*/*z* 494.9570, 496.9541, and 498.9563. Comparison of the spectroscopic data of **3** with those of **1** revealed a great similarity in their structures and the presence of an extra *O*-methyl group in **3** (δ_C 56.1 and δ_H 3.37 (s)), located at C-9 on the basis of the HMBC NMR correlations observed between the corresponding methoxy protons and C-9 and by the relative downfield shift of that carbon (δ_C 79.0). The relative configuration of **3** was mainly deduced by analysis of the TROESY spectrum and comparison with marilzabicycloallene A (**1**). On this basis, compounds **1** and **3** share the relative configuration determined to be 4*S**, 6*R**, 7*R**, 9*R**, 10*S**, 12*R**, 13*S** and 14*S**, as well as displaying the bromoallene unit with an *R* configuration.

Compound **4** was isolated as a colorless, amorphous solid. The molecular formula was deduced to be $C_{15}H_{19}Br_2Cl_3O_2$ by ESI-HRMS. The NMR spectra of **4** were very similar to those of **1** (Tables 1 and 2), indicating that **4** was also a representative of this new class of natural products and was identified as marilzabicycloallene D. The most significant differences were observed at the ^{13}C NMR chemical shift of C-9 and C-13, δ_C 60.1 and 63.0 in **4** vs δ_C 70.3 and 74.7 in **1**, respectively, revealing the replacement of both hydroxy groups of **1** by two chloride atoms in **4**, in agreement with the molecular formula and the relative intensities of isotope pseudomolecular peaks observed (see Supporting Information). The relative configuration of **4** was established through key ROE correlations and was found to be identical to **1** and **3**.

Biogenetically, obtusallenes II and IV are the first members of the obtusallene family to be encountered on the biosynthetic pathway via bromoetherification of a C_{15} precursor that, in turn, trace their origin to hexadeca-4,7,10,13-tetraenoic acid.[4,14] Obtusallenes II and IV have identical tetrahydrofuran cores and differ only in the absolute configuration at C-4 (*S* C_4-configuration for obtusallene II and *R* for obtusallene IV) and at the bromoallene unit (obtusallene II is *R* and obtusallene IV is *S*).[5,12] Guella and col.[3,12] have observed that several peculiarities emerged for both compounds revealing temperature-dependent NMR signals attributable to, at least, two equilibrating conformers by the flipping of the *trans* olefinic bond which mainly involves H-12β/H-13α to H-12α/H-13β inversion. This conformational phenomena and their relative conformer predominant populations were

(10) The absolute configuration of the bromoallene moiety was predicted according to the Lowe-Brewster's rule,[11] and confirmed by CD data through comparison with related compounds whose absolute configurations were assigned from X-ray diffraction data[12] (see Supporting Information).

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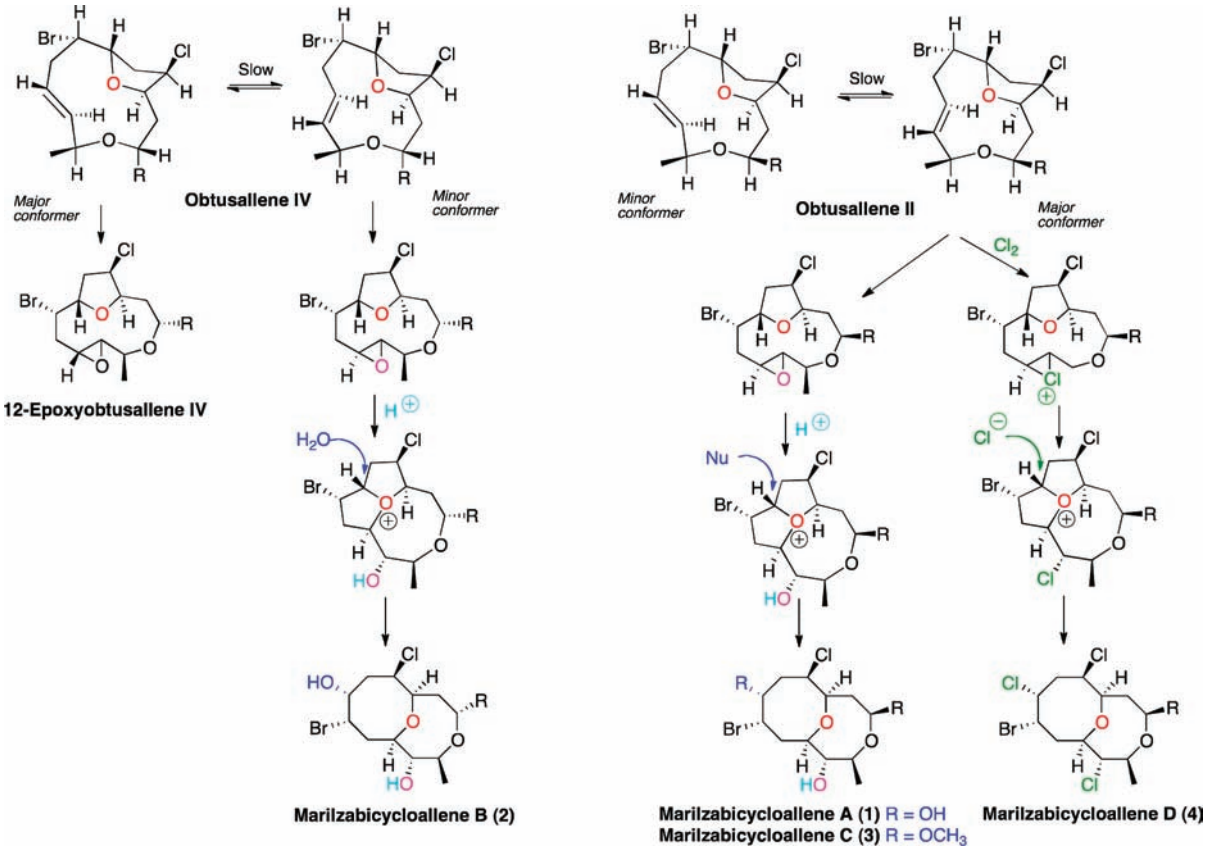
Scheme 1. Suggested Biogenetic Conversion of Obtusallenes II and IV into 12-Epoxyobtusallene IV and Marilzabicycloallenes A–D (**1**–**4**)



essential to trace the origin of marilzabicycloallenes A–D (**1**–**4**), as well as that of 12-epoxyobtusallene IV[9] recently isolated by us (Scheme 1). In a similar way to that proposed by Braddock for the biosynthesis of obtusallenes V–VII, we suggest that when obtusallenes II and IV show the closest transannular contact between the THF ether oxygen and C-12, an epoxide or chloronium ion formation in the *exo* face of C-12/C-13 double bond can occur. The H-12α/H-13*β* intermediates could evolve to generate subsequent tricyclic oxonium ions by transannular attack of the THF oxygen via a 5-*exo* process. The nucleophilic attack of either water or chloride at C-9 leads directly the [5.5.1]bicyclotridecane ring system observed in the marilzabicycloallenes, where all the stereochemistry is correctly set.

Metabolites **1** and **2** were evaluated for their in vitro antiproliferative activity against six human tumor cell lines: A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (nonsmall-cell lung), T-47D (breast), and WiDr (colon).[15] None of the metabolites showed significant activity against the above- mentioned cancer cell lines ($GI_{50} > 10\ \mu g/mL$).

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Supporting Information Available. Experimental procedures and copies of ^{1}H, ^{13}C and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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