JOURNAL OF NATURAL PRODUCTS

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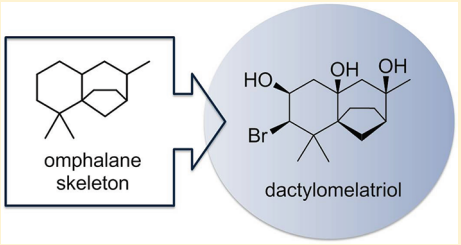
Dactylomelatriol, a Biogenetically Intriguing Omphalane-Derived Marine Sesquiterpene

Ana-R. Díaz-Marrero, José M. de la Rosa, Inmaculada Brito, José Darias, and Mercedes Cueto*

Instituto de Productos Naturales y Agrobiología (CSIC), Avenida Astrofísico F. Sánchez, 3, 38206 La Laguna, Tenerife, Spain

Supporting Information

ABSTRACT: Dactylomelatriol (**1**), obtained from the sea hare *Aplysia dactylomela*, is the first naturally occurring omphalane-derived sesquiterpene from the marine environment. From this species the known chamigrene and modified bisabolene sesquiterpenes **2**–**6** were also isolated. The structure and relative configuration of **1** were established by spectroscopic evidence. Its chemical structure is related to omphalic acid, the unique terrestrial-derived omphalane sesquiterpene isolated from a liverwort. A biogenetic route for this compound is proposed. The antimicrobial activities of compounds **1**–**6** were evaluated against a panel of microorganisms.



Sesquiterpenes are widespread in algae, marine invertebrates, microorganisms, higher plants, liverworts, mosses, arthropods, and fungi.[1] Studies on natural products chemistry reveal that some compounds are only produced by certain species, therefore conferring upon them a chemical signature.[2] In the marine environment, secondary metabolites possessing a chamigrene skeleton are characteristic of red algae of the genus *Laurencia*[3] and the sea hares grazing on them. Furthermore, metabolites derived from this skeleton have also been found in lower terrestrial plants of the class Hepaticae[4] (liverworts), in higher plants,[5,6] and, very recently, in a Basidiomycetous fungus.[7]

We have described some new chamigrenes[8] as well as regular, rearranged, and degraded bisabolene-type metabolites[9] from *Aplysia dactylomela* collected off the coast of La Palma (Canary Islands). In the present study we report on the isolation of compounds **1**–**6** (Figure 1) from *A. dactylomela* collected off the southern coast of La Gomera (Canary Islands). Compound **1** represents the first example of a marine-derived omphalane sesquiterpene, which is belived to derive from a chamigrene precursor. The only other metabolite with this basic skeleton is omphalic acid (**7**) from the liverwort *Omphalanthus filiformis*.[10] The known compounds isoobtusol (**2**), elatol (**3**), obtusol (**4**), caespitenone (**5**), and caespitane (**6**), were characterized by comparison of their NMR spectroscopic data with those for compounds previously isolated from *Laurencia*[2,11,12] and/or *Aplysia* species.[8,9,13]

Dactylomelatriol (**1**) was obtained as a colorless oil. Its EIMS spectrum showed peaks at *m*/*z* 332/334 $[M]^+$ with relative intensities that indicated the presence of a bromine atom and a molecular formula $C_{15}H_{25}BrO_3$ (HREIMS). The NMR experiments of **1** were performed in C_6D_6 to obtain better resolution of the methylenic signals and to avoid overlapping of the D_2O exchangeable hydroxy protons observed in the ^1H NMR spectrum in $CDCl_3$. The ^{13}C NMR spectrum of **1** (Table 1) indicated the presence of 15 carbons in the molecule whose multiplicities were determined by DEPT spectroscopic data as follows: three methyl groups, five methylenes, three methines (two bearing a heteroatom), and four sp^3 quaternary carbons (two bearing a heteroatom). The ^1H NMR spectrum (Table 1) showed signals for two methines geminal to a heteroatom at δ 3.73 (d, J = 2.5 Hz) and 3.87 (dddd, J = 4.5, 3.1, 3.1, 3.1 Hz) and three D_2O exchangeable signals indicative of the presence

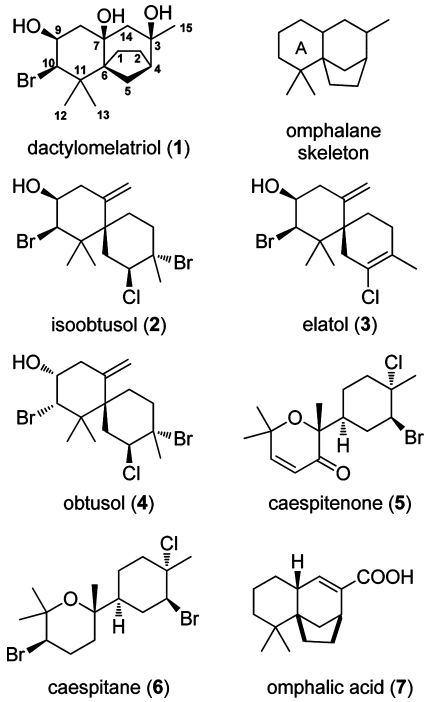


Figure 1. Sesquiterpenes **1**–**6** from *A. dactylomela* and **7** from a liverwort.

Received: October 18, 2011
Published: January 5, 2012

Table 1. ^{1}H NMR,^{13}C NMR, and HMBC Data of Compound 1 [^{1}H 500 MHz, δ ppm, ^{13}C 125 MHz C_6D_6]

position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]
1	27.7, CH_2	α: 0.61, m	5, 6, 7
		β: 1.36, m	
2	27.3, CH_2	1.09, m	
		1.36, m	
3	73.1, C		
4	46.9, CH	1.83, dd (4.8, 4.8)	6, 14
5	31.2, CH_2	*endo*: 2.44, ddd (12.2, 1.9, 1.9)	1, 7
		exo: 1.09, m	
6	54.6, C		
7	77.5, C		
8	39.1, CH_2	α: 1.26, dd (14.9, 3.7)	6, 7, 9
		β: 1.66, dd (14.9, 3.1)	
9	73.6, CH	3.87, dddd (4.5, 3.1, 3.1, 3.1)	10
10	71.7, CH	3.73, d (2.5)	11, 12, 13
11	40.6, C		
12	23.9, CH_3	1.43, s	6, 10, 11, 13
13	28.4, CH_3	0.87, s	6, 10, 11, 12
14	46.2, CH_2	α: 0.94, d (14.4)	3, 4, 6, 7, 8
		β: 1.20, dd (14.4, 1.7)	
15	27.4, CH_3	1.00, s	3, 4, 14
3-OH		3.02, s	3, 4, 15
7-OH		4.34, s	7, 8, 14
9-OH		2.93, d (4.8)	8, 9

[a]HMBC correlations are from the proton(s) stated to the indicated carbon.

of three hydroxy groups in the molecule. Upfield signals for three methyl groups at δ 0.87 (s), 1.00 (s), and 1.43 (s) and 11 protons between δ 2.44 and 0.61 account for the remaining aliphatic protons of the molecule. From the molecular formula and the spectroscopic data it can be deduced that the molecule must be tricyclic, and the presence of only three methyl groups indicates that one of the four methyl groups, expected for a regular sequiterpene carbon skeleton, must be involved in one of the rings.

Overlapping signals for two methylene protons at δ 1.09 m and 1.36 m have restricted the unambiguous interpretation of the ^{1}H−^{1}H-COSY experiment to two discrete spin systems: H_2-8−H-10 and H_2-1−H_2-2 (Figure 1). The HSQC and HMBC data were used to confirm these fragments and to establish their connectivities. The three-bond correlations of H_3-12 and H_3-13 to the respective carbons C-13 and C-12, as well as their correlations to both C-10 and C-11, allowed the placement of a *gem*-dimethyl group at C-11. HMBC correlations of H_3-12, H_3-13, and H_2-14 with C-6 and correlation of H_2-14 with C-8 defined ring A. Also, HMBC correlations of H_2-1 (δ 0.61, m) with C-5, C-6, and C-7 allowed us to attach C-1 and C-5 to the spiro carbon C-6. These correlations, in addition to H_3-15 with C-3, C-4, and C-14, and H-4 with C-6, allowed the establishment of the connectivities for the remaining five- and six-membered rings of the molecule, and because the linkage C-1/C-6 was secured, the COSY correlations between δ 1.83 (dd, J = 4.8,4.8 Hz) and 1.36 (m) allowed the connection of C-2 and C-4.

Finally, signals of three hydroxy protons at δ 3.02 (s), 4.34 (s), and 2.93 (d, 4.8) were correlated with C-3, C-7, and C-9, respectively, indicating that the bromine atom is attached to C-10 and that C-3, C-7, and C-9 bear hydroxy groups.

The relative configuration of compound **1** was assigned on the basis of NOESY experiments (Figure 2). The NOEs

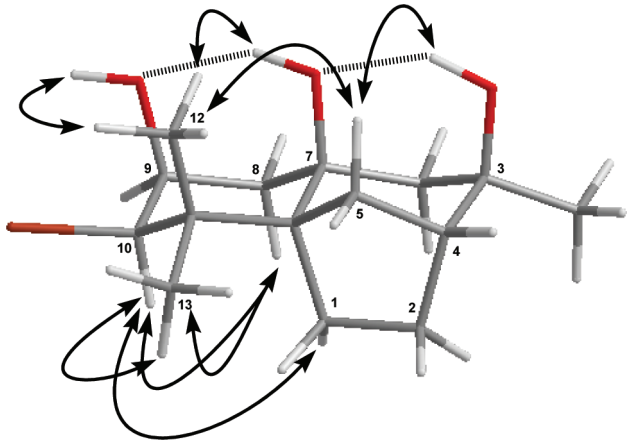


Figure 2. Minimized structure and selected NOEs for dactylomelatriol (**1**).

observed for H-10 with H-8α, H-1α, and H_3-13, as well as the NOEs observed between H_3-12 and OH-9 and OH-7, fixed the configuration of ring A, indicating that the hydroxy groups at C-9 and C-7 are on the same side of the molecule. The configuration at C-3 was established by the NOEs observed between H-5_{endo} and H_3-12 and OH-3.

Molecular mechanics energy minimization[14] of the proposed structure for **1** was performed. The minimized structure of **1** (Figure 2), which shows intramolecular hydrogen bonds, has the appropriate interatomic distances for the NOEs observed. Therefore, the relative configuration of dactylomelatriol is as depicted in Figure 2.

Seaweeds of the genus *Laurencia*, lower terrestrial plants (liverworts), and higher plants in the Cupressaceae family feature the exclusive ability to biosynthesize tricyclic sesquiterpene derivatives by internal cyclization of a chamigrene precursor. However, it is noteworthy that the chemical mechanisms of the cyclization can follow different courses that appear to be dependent on the producing organism. For example, marine organisms have provided gomerane[15] and rhodolaurane-derived[16] metabolites that evolve exclusively from cyclization of the exocyclic methylene C-14 of a β-chamigrene precursor, Figure 3. On the contrary, from the terrestrial environment, the central isoprenic C-14 carbon of the related chamigrene-derived tricyclic skeletons such as junicedrane, chamipinane (Cupressaceae),[5,6] and myltaylane[17] (liverworts) remains as an intact methyl group (Figure 3).

Our discovery of the naturally occurring dactylomelatriol (**1**) establishes that the omphalane skeleton is also present in the marine environment. Hence, the coexistence of omphalane derivatives in both marine and terrestrial environments makes their biogenesis intriguing, because the tricyclic network of the naturally occurring omphalic acid (**7**), isolated from the liverwort *Omphalanthus filiformis*,[10] revealed that the central isoprenic C-14 does not remain as an intact methyl group but forms a part of a ring (Figure 1).

In light of these observations, we believe that the formation of dactylomelatriol must first involve the formation of the rhodolaurane skeleton. Isolation of the C-7/C-14 epoxyobtusol from *Laurencia obtusa*[18] suggests a possible route to this intermediate and allowed us to propose the biogenesis of the

dx.doi.org/10.1021/np200845f | *J. Nat. Prod.* 2012, 75, 115−118

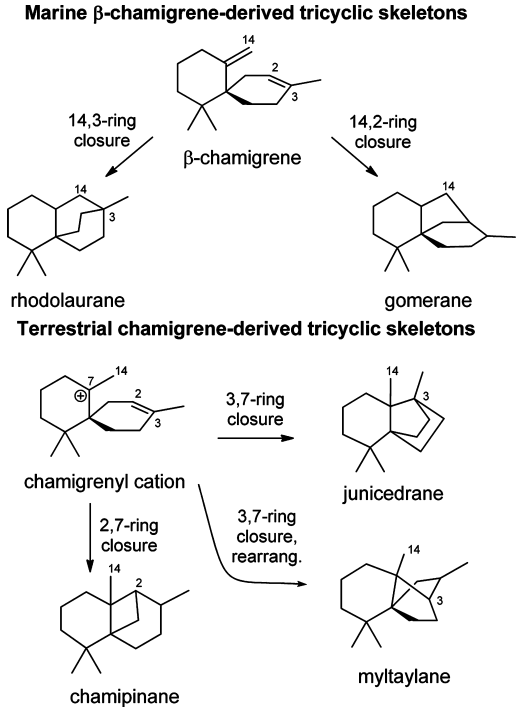


Figure 3. Marine and terrestrial tricyclic chamigrene-derived skeletons.

tricyclic network of dactylomelatriol, in the sequence depicted in Figure 4. An epoxide precursor formed from deschloroelatol

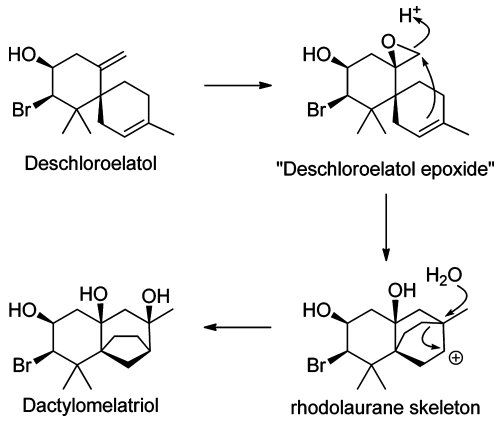


Figure 4. Possible biogenesis of dactylomelatriol.

could be protonated, inducing internal cyclization to form a rhodolaurane skeleton intermediate, which could undergo a 1,2-shift to generate the omphalane ring system. Quenching of the resulting tertiary cation at C-3 with water would form dactylomelatriol (**1**).

Because mollusks of the genus *Aplysia* feed on algae of the genus *Laurencia* and the only known compounds possessing a rhodolaurane skeleton have been isolated from alga of this genus,[16] it seems possible that *Laurencia* is the true producer of **1** or at least the producer of its precursor. Therefore, we are planning to analyze extracts of algae of the genus *Laurencia*, collected at the same location as the *Aplysia*, in order to ensure the parent producer of dactylomelatriol.

Compounds **1**–**6** were tested for their antimicrobial activity. Only compounds **3** and **4** showed weak antibacterial activity against *B. cereus* (MIC > 50 μg/mL). Compound **4** was also found to be weakly antimycotic against *Candida albicans* (MIC > 50 μg/mL).

EXPERIMENTAL SECTION

General Procedures. Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter using a Na lamp at 25 °C. IR spectra were recorded on a Perkin-Elmer 1650/FTIR spectrometer. ^{1}H NMR and ^{13}C NMR, HSQC, HMBC, and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. All ^{13}C and ^{1}H NMR spectra were internally referenced to the residual solvent signal (C_6D_6: δ_C 128.0 ppm, δ_H 7.16 ppm). Two-dimensional NMR spectra were obtained using the standard Bruker software. EIMS and HRMS data were obtained on a Micromass Autospec spectrometer. HPLC separations were performed with a Hewlett-Packard 1050 (Jaigel-Sil semipreparative column, 10 μ, 20 × 250 mm) with hexane–EtOAc mixtures. Size-exclusion chromatography used Sephadex LH-20 as the stationary phase and hexane–MeOH–CH_2Cl_2 (3:1:1) as the solvent system. The spray reagent used to develop TLC plates was H_2SO_4–H_2O–AcOH (1:4:20).

Biological Material. *Aplysia dactylomela* specimens (41) were collected off the south coast of La Gomera (Canary Islands, Spain) at 1.5 m depth. Specimens were dissected, and their digestive system and the mantle were separated and analyzed independently. A voucher specimen is deposited at the Museum of Natural Sciences of Tenerife (deposit no. BM/005359-MO/000275).

Extraction and Isolation. *A. dactylomela* digestive glands were extracted with acetone at room temperature. The extract was concentrated to give a dark green residue (121.0 g) that was partitioned with CH_2Cl_2–H_2O. The resulting fraction of CH_2Cl_2 (19.3 g) was then submitted to flash chromatography on Si gel to give fraction D (hexane–EtOAc (95:5) (5.4 g)), which after gel filtration chromatography and HPLC yielded the known compounds elatol (**3**, 105.5 mg) and caespitane (**6**, 95.1 mg). From fraction E (hexane–EtOAc (90:10) (3.1 g)) after gel filtration chromatography and HPLC were isolated the known compounds isoobtusol (**2**, 152.3 mg) and caespitenone (**5**, 170.8 mg). The fraction eluted with hexane–EtOAc (7:3) (653.1 mg) was chromatographed by gel filtration and HPLC (Jaigel-sil column 20 × 250 mm, flow rate 4.5 mL/min, hexane–EtOAc (1:1)) to give the new sesquiterpene **1** (2.7 mg).

A. dactylomela mantles were extracted and processed following the same scheme. From the extract of the mantle compound **1** (3.1 mg), elatol (**3**, 39.6 mg), obtusol (**4**, 22.2 mg), and ceaspitenone (**5**, 1.0 mg) were isolated. No isoobtusol (**2**) or caespitane (**6**) was detected.

Dactylomelatriol (1): colorless oil; $[\alpha]^{25}_D = -22$ (c 0.14, $CHCl_3$); IR (film) ν_{max} 3360, 2910 cm^{-1}; ^{1}H and ^{13}C NMR (C_6D_6), see Table 1; EIMS m/z 332/334 $[M]^+$ (1, 1), 314/316 $[M - H_2O]^+$ (6, 6), 253 $[M - Br]^+$ (3), 204/206 (87, 84), 129 (100); HREIMS $[M]^+$ 332.0994 (calcd for $C_{15}H_{25}BrO_3$, 332.0987), $[M - H_2O]^+$ 314.092 (calcd for $C_{15}H_{23}BrO_2$, 314.0881), $[M - Br]^+$ 253.1802 (calcd for $C_{15}H_{25}O_3$, 253.1804).

Antimicrobial Activity. Antimicrobial activity (within the range 10–50 μg/mL) was determined by the broth macrodilution method against the following strains obtained from the Spanish Collection of Type Cultures (CECT; Faculty of Biological Sciences, University of Valencia, Spain) and the American Type Culture Collection (ATCC, USA): *Staphylococcus aureus* (ATCC 6538), *Salmonella* sp. (CECT 456), *Klebsiella pneumonia* (ATCC 23357), *Escherichia coli* (ATCC 9637), *Bacillus cereus* (ATCC 21772), *Proteus mirabilis* (CECT 170), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (SC5314) as described elsewhere.[19] In the case of *C. albicans* the tryptic soy medium was replaced by the non-filament-inducing medium YPD [2% (w/v) Bacto peptone, 1% (w/v) yeast extract, and 2% (w/v) glucose].

dx.doi.org/10.1021/np200845f | J. Nat. Prod. 2012, 75, 115–118

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR, HSQC, and HMBC spectra of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +34 922 252 144. Fax: +34 922 260 135. E-mail: mcueto@ipna.csic.es.

■ ACKNOWLEDGMENTS

This work was supported by the Ministerio de Ciencia e Innovación (BIO2007-61745, SAF2009-08039, SAF2010-16858) and DGUI Gobierno de Canarias (PIO42005, PUB2005/030). We gratefully acknowledge A. Corrales for her technical support of this project.

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