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Aplysiadiol from *Aplysia dactylomela* suggested a key intermediate for a unified biogenesis of regular and irregular marine algal bisabolene-type metabolites

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Abstract—The rearranged compound **1**, the regular **2** and **3** and the degraded **4** are novel bisabolene-type metabolites that along with the known compounds caespitol, 8-acetylcaespitol, caespitane, caespitenone, laucapyranoid A, and furocaespitane have been isolated from the sea hare *Aplysia dactylomela*. The structures of these compounds were determined on the basis of spectroscopic evidence. The irregular network of **1** suggested that the halogenated epoxide **6** is a key intermediate for a unified biogenetic pathway of naturally occurring marine algal bisabolene-type metabolites.

1. Introduction

Molluscs of the genus *Aplysia* are one among the best studied of all marine invertebrates, serving as a model system for neurological studies.[1] *Aplysia dactylomela* (Opisthobranchia: Anaspidea) like other sea hares thrive on algae, acquiring and storing many algal metabolites in their digestive gland.[2] This species alone sequesters some 80 different secondary metabolites from its algal diet,[3] but their function is a subject of debate because the dynamics of metabolites of sea hares is poorly understood.[4] *Laurencia caespitosa*, a red alga common in the diet of *A. dactylomela* from the Canary Islands, was the first source[5] of a marine algal bisabolene-type sesquiterpenoid, and since then a number of metabolites belonging to this skeletal class has been found in other taxa including sponges, nudibranchs, octocorals, and, more recently, microorganisms.

From a survey on marine bisabolene metabolites interesting analogies can be observed regarding structural and/or functional features. For example, all bisabolene derivatives from sponges can be grouped into two structural classes. One class of metabolites bear an aromatic ring[6–14] related to (+)-curcuphenol and the other features a diverse nitrogenous substitution pattern in a non-aromatic network[15–21] (Fig. 1). Moreover, all bisabolene compounds from octocoral

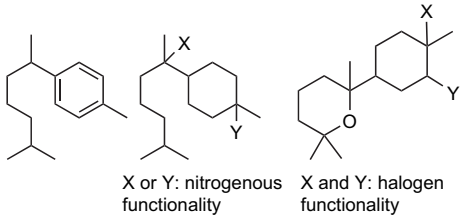

Figure 1. Representative structural classes of bisabolene-type metabolites from diverse marine taxa.

are aromatic, sharing with sponges some identical but, interestingly, antipodal metabolites related to (−)-curcuphenol.[22–25] Recently discovered microbial bisabolene compounds[26] were also related to the curcumene skeleton whereas bisabolene products from red algae are mostly characterized, unlike the other taxa, by high halogen content.[5,27–29]

A. dactylomela has proved to be a rich source of structural variety of bisabolenic metabolites, depending on where they are located.[5,30–33] We now report on the discovery of new bisabolene derivatives **1–4** from this species, along with the previously characterized caespitol,[5] furocaespitane,[27] laucapyranoid A,[28] 8-acetylcaespitol,[32] caespitane,[28,32] and caespitenone[28,32] (Fig. 2). Most of the compounds reported here are halogenated, following the general pattern of algal bisabolene sesquiterpenoids, **1** and **4** being a rearranged and a degraded bisabolene skeleton derivative, respectively.

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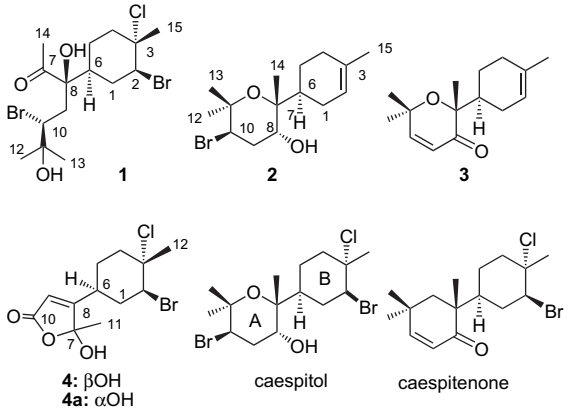


Figure 2. Metabolites from *A. dactylomela*.

2. Results and discussion

A. dactylomela was collected off the SW coast of La Palma (Canary Islands). From the crude extracts the new compounds **1**–**4** were isolated after gel filtration on Sephadex LH-20 chromatography followed by successive HPLC.

Aplysiadiol **1** was a colorless oil and its NMR spectroscopic data are summarized in Table 1. Its EIMS spectrum showed peaks at *m/z* 413/415/417/419 with relative intensities suggestive of one chlorine and two bromine atoms, which correspond to the empirical formula $C_{14}H_{20}O_2ClBr_2$ $[M-Me-H_2O]^+$ (HREIMS). Absorption for a carbonyl and a hydroxyl group at 1722 and 3448 cm^{-1}, respectively, were observed in its IR spectrum. The ^{13}C NMR and DEPT spectra of **1** showed the presence of 15 carbon signals assigned to $4\times CH_3$, $4\times CH_2$, $3\times CH$ (two bearing bromine), and four quaternary carbons (one ketone). The ^1H NMR spectrum displayed signals for two protons geminal to bromine at δ 3.67 (dd, J=2.1, 9.5 Hz) and δ 4.27 (dd, J=4.3, 12.8 Hz); eight methylene protons between δ 2.55 and 1.60; a methine proton at 1.84 (br s); and four methyl groups [δ 2.30 (s), δ 1.68 (s), δ 1.35 (s), δ 1.32 (s)]. According to the degree of unsaturation, the molecular formula of **1** expressed a monocyclic network.

Connectivity information obtained from COSY, HSQC, and HMBC experiments unambiguously determined a cyclohexyl moiety with a halogen substitution pattern identical with that in the corresponding ring B of caespitol (Fig. 2). The complementary half of the molecule attached to the ring is a highly functionalized open chain where the regiochemistry of a carbinolic bromohydrin, involving a gem-dimethyl group, becomes readily apparent by the mutual HMBC correlations C-13/H_3-12, C-12/H_3-13 and those with H-10 and C-11, respectively. The COSY correlation of H-10 with a remaining methylene placed a carbinolic methyl ketone at C-8, establishing the planar structure of **1** as an irregular sesquiterpene.

The coupling constant of H-2 (J=4.3, 12.8 Hz) as well as the ^{13}C-chemical shifts of C-2, C-3, and C-15 are in agreement with the regiochemistry and configuration of a vicinal chlorobromo system on a cyclohexane ring with a chair conformation such as that of caespitol (Table 1). An equatorial disposition of the side chain residue on the ring was

Table 1. NMR data of compounds **1**–**4** and caespitol [500 MHz, δ ppm, (J) Hz, $CDCl_3$]

#	1		2		3		4		Caespitol	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.80 (m), 1.98 (m)	35.7	1.73 (m), 1.99 (m)	25.9	1.87 (m), 2.09 (m)	25.1	2.00 (m), 2.00 (m)	39.8	α: 2.21 (m), β: 1.57 (ddd, 12.6, 12.6, 12.6)	36.3
2	4.27 (dd, 4.3, 12.8)	62.0	5.32 (br s)	119.8	5.37 (br s)	120.6	4.38 (dd, 4.1, 12.4)	60.8	4.34 (dd, 4.5, 12.4)	63.7
3	—	71.0	—	134.5	—	133.9	—	70.2	—	72.0
4	α: 2.06 (m), β: 2.45 (ddd, 3.4, 3.4, 13.7)	42.2	1.90 (m), 2.03 (m)	30.9	1.89 (m), 2.00 (m)	30.6	α: 2.16 (ddd, 3.9, 13.7, 13.7), β: 2.48 (ddd, 3.4, 3.4, 13.9)	42.2	α: 2.00 (ddd, 3.8, 13.5, 13.5), β: 2.35 (ddd, 3.3, 3.3, 13.5)	42.9
5	1.76 (m), 1.60 (m)	23.8	1.19 (m), 1.99 (m)	22.5	1.47 (m), 1.56 (m)	24.0	1.50 (m), 1.50 (m)	28.8	1.18 (m), 1.82 (m)	22.7
6	1.84 (br s)	46.3	1.70 (br s)	41.4	2.07 (m)	41.6	2.53 (dddd, 3.2, 3.2, 12.0, 12.0)	36.9	1.85 (m)	45.8
7	—	212.7	—	77.8	—	81.9	—	106.8		77.2
8	—	82.7	—	71.4	—	199.9	5.80 (s)	170.4	3.52 (dd, 2.3, 3.3)	70.8
9	a: 2.55 (dd, 2.1, 15.6), b: 2.27 (dd, 9.5, 15.6)	39.4	3.52 (ddd, 3.6, 3.6, 6.2) α: 2.47 (ddd, 2.3, 13.6, 13.6), β: 2.26 (ddd, 4.1, 4.1, 13.9)	35.8	5.92 (d, 10.4)	122.8	—	116.4	α: 2.17 (m), β: 2.46 (ddd, 2.3, 13.4, 13.4)	36.2
10	3.67 (dd, 2.1, 9.5)	60.4	4.33 (dd, 4.1, 13.2)	53.9	6.80 (d, 10.4)	154.7	—	172.6	4.28 (dd, 4.2, 13.2)	53.2
11	—	72.9	—	75.3	—	71.2	1.71 (s)	24.1	—	75.4
12	1.35 (s)	27.6	1.32 (s)	31.2	1.36 (s)	30.0	1.75 (s)	24.1	1.26 (s)	31.0
13	1.32 (s)	25.5	1.40 (s)	24.3	1.41 (s)	29.7	—	—	1.32 (s)	24.0
14	2.30 (s)	26.6	1.13 (s)	19.2	1.30 (s)	23.4	—	—	1.08 (s)	19.9
15	1.68 (s)	22.8	1.62 (s)	23.4	1.62 (s)	23.4	—	—	1.63 (s)	24.3
7-OH							1.56 (br s)			
8-OH	3.85 (s)		1.84 (d, 6.4)						2.43 (br s)	

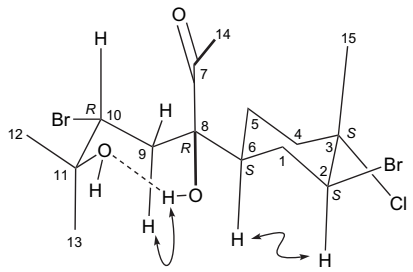
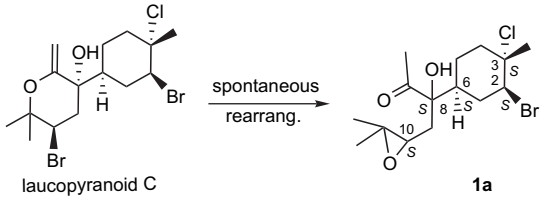


Figure 3. Selected NOEs of compound **1**.

established by the strong NOE observed between H-2 and H-6 (Fig. 3).

The energy-minimized conformation of **1** deduced by molecular mechanics[34] is shown in Figure 3. This conformer showed a hydrogen bond between the hydroxyl groups so that the proton of the hydroxyl at C-8 and the trans-diaxial proton at C-9 are at a suitable interatomic distance to allow the observed NOE between them. For the acyl group at C-8 an *R* configuration is given to allow an atomic cluster compatible with the H_2-9/H-10 *J*-coupling and the observed NOE. This point has been further reinforced by comparison of the NMR data of **1** with those of a related compound **1a** generated by spontaneous rearrangement of laucopyranoid C and whose absolute configuration was established by X-ray crystallography.[28] Whereas the ^{13}C chemical shifts of the carbons of the cyclohexane ring are almost identical in both compounds, the inversion of the configuration at C-8 of **1** produced a variation in the chemical shift of this carbon and the comparable vicinal carbons. For ^{13}C chemical shifts of **1a** see the experimental part. This spectroscopic correlation allowed us to propose for **1** the 2*S*, 3*S*, 6*S*, 8*R*, 10*R* configuration depicted in Figure 3.



Deschlorobromo caespitol **2** was obtained as a white powder. The EIMS spectrum showed peaks at *m/z* 316/318, with relative intensities for one bromine atom. NMR data coupled with the $[M]^+$ peak in the HREIMS of **2** suggested a molecular formula $C_{15}H_{25}O_2Br$ with three degrees of unsaturation indicating that the molecule is bicyclic. The ^{13}C NMR and DEPT spectra of **2** (Table 1) showed the presence of 15 carbon signals assigned to $4\times CH_3$, $4\times CH_2$, $4\times CH$ (one olefinic and two bearing heteroatoms), and three quaternary carbons (one olefinic and two bearing heteroatoms). The following ^1H NMR signals were observed: one olefinic proton at δ 5.32 (br s); two protons geminal to heteroatom [δ 4.33 (dd, J=4.1, 13.2 Hz), δ 3.52 (ddd, J=3.6, 3.6, 6.2 Hz)]; one methine proton at δ 1.70 (br s); eight methylene protons between δ 2.03 and 1.19; four methyl groups [δ 1.62 (br s), δ 1.40 (s), δ 1.32 (s), δ 1.13 (s)], and one D_2O interchangeable proton at δ 1.84 (d, J=6.4 Hz).

Connectivity information obtained from COSY, HSQC, and HMBC as well as NOESY experiments secured an oxane ring moiety bearing the same heteroatoms, regiochemistry and relative configuration as those of comparable atoms of ring A of caespitol (Table 1). The remaining two degrees of unsaturation correspond to a trisubstituted double bond in a residual cyclohexene ring, which suggests that the typical chlorobromo system of ring B of caespitol was lost in compound **2**. On the other hand, it has been reported that the $LiAlH_4$ reduction of caespitol yielded a compound[5] with same planar structure as **2**. The ^{13}C NMR chemical shifts reported later[35] for this synthetic compound were very similar to those of **2** except for the corresponding C-3 and C-15 carbon atoms ($\Delta_{\delta C\text{-}3}$=5.6, $\Delta_{\delta C\text{-}15}$=10 ppm). Since ring A of both natural and synthetic compounds are identical in all respects, and because such differences are improbable for a putative diastereomer at C-6, we suspect these values were mistaken or incorrectly assigned. In order to corroborate this point, caespitol was efficiently dehalogenated by reaction with Zn–NaI in DMF at 115 °C to give a compound identical with that depicted in **2**. Thus, the correct NMR data of **2** are assigned in Table 1.

Deschlorobromo caespitenone **3** was isolated as an oil. NMR spectroscopic data coupled with a molecular ion peak at *m/z* 234 $[M]^+$ (HREIMS) suggested a molecular formula of $C_{15}H_{22}O_2$ indicating five degrees of unsaturation. The ^1H and ^{13}C NMR data of **3** are similar to those of the known compound caespitenone,[28,32] also isolated in this work. The main difference is that the chlorobromo system characteristic of ring B of caespitenone has been lost in **3**, leading to the corresponding cyclohexene ring, which is in accord with the degrees of unsaturation. Thus, **3** is the dehalogenated derivative of caespitenone. This was corroborated by oxidation–dehydration of compound **2** with Jones reagent to give a compound identical with that depicted in **3**.

Since the optical rotation of caespitol isolated in this study is coincident with that previously reported,[28] the chemical transformation of caespitol into compounds **2** and **3** allowed us to establish their absolute streochemistries as 6*S*,7*R*,8*R*,10*R* and 6*S*,7*R*, respectively.

Furocaespitanelactol **4** was isolated as a white powder. Its EIMS spectrum showed peaks at *m/z* 322/324/326 with relative intensities suggestive of one chlorine and one bromine atom, which correspond to the empirical formula $C_{12}H_{16}O_3ClBr$ $[M]^+$ (HREIMS). The molecular formula of **4** resembled that of furocaespitane, a degraded bisabolene-type derivative previously isolated by us from *L. caespitosa*[27] and also now isolated in this work from *A. dactylomela*.

Oxidation of furocaespitane with *m*-chloroperbenzoic acid has been reported to give an epimeric mixture[27] at C-7 of butenolides **4** and **4a** (Fig. 2). The spectroscopic data of our single naturally occurring compound indicates that its structure is a lactol identical to one of the epimers present in the synthetic mixture. The energy-minimized conformation of both epimers **4** and **4a** deduced by molecular mechanics is shown in Figure 4.

The orthogonal joining of the lactone and cyclohexane rings placed the methyl group of the respective carbinols of each epimer at such a similar distance from H-6 that it does not allow us to discern to which one of the epimers the NOE

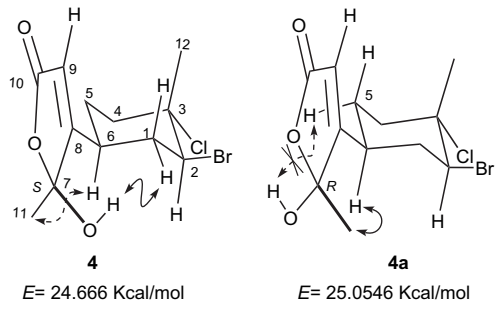


Figure 4. Selected NOEs of **4** and **4a**.

observed between CH_3-11 and H-6 corresponds. However, the interatomic distance between the hydrogen of the hydroxyl group and the equatorial proton H_2-1 of **4** measured by the program is 2.446 Å, whereas in the epimer **4a** the distance to H-5_{eq} is 3.111 Å, which appears to be too large for a clear NOE to be expected. Thus, the observed NOE in the natural compound was assigned to the epimer with an *S* stereochemistry at C-7. Because the remaining chiral centers of the compound are identical with the corresponding centers of furocaespitane, the stereochemistry of the degraded bisabolene metabolite was assigned as depicted in **4** (Fig. 4).

On standing, **4** isomerizes to **4a** until a 1:1 equilibrium mixture is reached (Fig. 5). This is the first time that **4** has been isolated as a natural product. Due to the lack of ^{13}C NMR data and assigned ^{1}H NMR chemical shifts, we provide complete NMR data of the natural compound **4** in Table 1 and those of **4a** in the experimental part.

A bisabolyl cation is expected to be converted into the possible precursor of the naturally occurring algal bisabolene derivatives, the true producer of this type of metabolite found in *A. dactylomela*. It appears that in this process the trienic system of the bisabolene is sequentially oxidized, starting with chlorobromo addition to the endocyclic double bond. This seems to be supported by finding the natural halogenated addition products puertitols A and B[36] by stabilization of the intermediate **5** (Fig. 6).

Although **6**, a putative derivative from a second oxidation step of the central double bond of the halogenated bisabolol **5**, is unknown at present, it can be postulated as a precursor in the biogenesis of these compounds. The nascent intermediate **6** rearranges, by 1,2-migration of the bond connecting both the linear and cyclic halves induced by epoxide ring opening, to **7**. The transformation of **6**→**7** is a key step to account for naturally occurring marine algal irregular and degraded bisabolene-type network metabolites.

From **7**, and in virtue of the biogenetically interconvertible **8**↔**9**, alternative path a and/or path b may provide a non-regular network **8** (Box **A**) from which laucopyranoid A could be generated by stabilization of carbocation **8**.

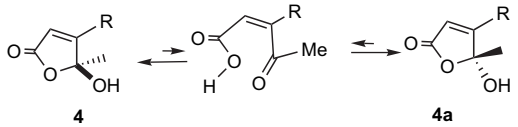


Figure 5. Equilibrium leading to a mixture of epimers at C-7.

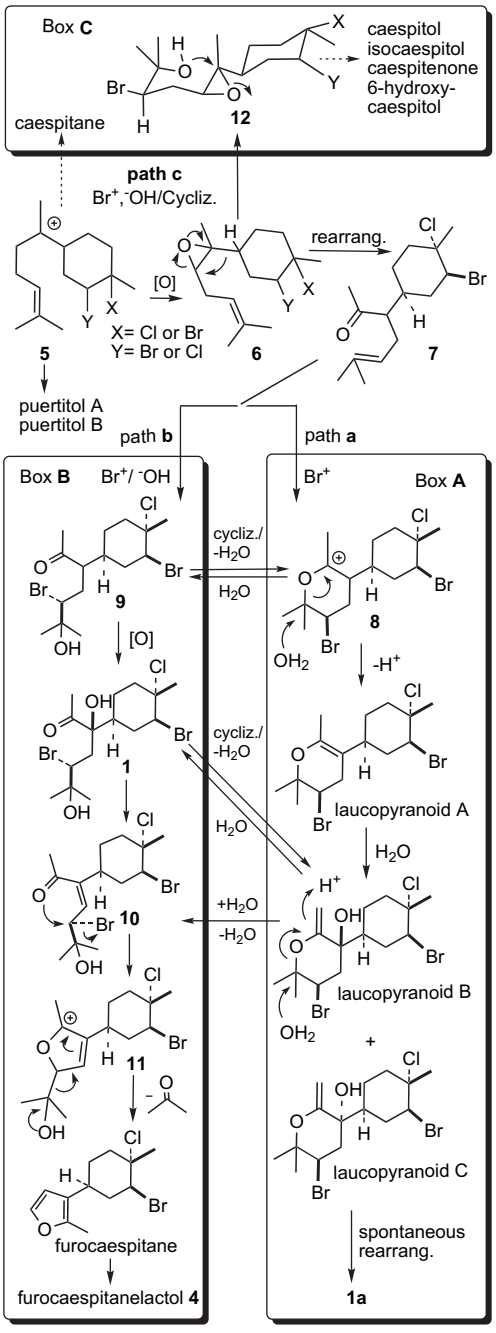


Figure 6. Unified biogenesis for marine algal bisabolene-type metabolites.

Hydration of laucopyranoid A may afford the epimeric laucopyranoid B and laucopyranoid C. Whereas laucopyranoid C spontaneously rearranges to **1a**, laucopyranoid B as well as aplysiadiol **1**, derived from extensive oxidation of **7**, Box **B**, may evolve to degraded furocaespitane and related lactol **4** following path **b** indicated in Figure 6. This route involves dehydration of **1** and/or hydration/dehydration of laucopyranoid B to a conjugated ketone **10**, formation of a furane ring by intramolecular displacement of the bromine atom and, finally, trapping of the resulting carbocation **11** by loss of the terminal isopropyl carbinol as acetone.

On the other hand, the key precursor **6** may evolve by oxidizing the remaining olefin, the terminal isopropylidene, to a fully oxidized bromohydrin derivative **12** (Box **C**) from

which the regular sesquiterpenoid caespitol, and related compounds such as isocaespitol,[37] caespitenone,[32] and 6-hydroxycaespitol[28] may originate through path **c**. Cyclization of the $Br^+/^-OH$ addition product of **5** will produce caespitane. Thus, we propose a unified pathway for the biogenesis of regular, irregular rearranged, and degraded bisabolene-type metabolites isolated from red algae of genus *Laurencia* and the sea hares that thrive on them.

Until now all reported marine algal bisabolene-type metabolites bear a vicinal chlorobromo system on their carbocyclic ring. However, the corresponding dehalogenated derivatives of caespitol and caespitenone, compounds **2** and **3**, respectively, have been found for the first time as naturally occurring metabolites in *A. dactylomela*, suggesting that these compounds have been chemically transformed by the mollusc to store them as less toxic compounds. This statement is supported by the recent finding that the naturally occurring deschloroelatol found in *A. dactylomela* is less toxic than the co-occurring chlorinated elatol.[33] This resembles the known[38,39] strategy used by *A. dactylomela* by which some acquired algal metabolites are occasionally acetylated to decrease the toxicity[33] of the corresponding alcohols.

Furthermore, the butenolide unit is widespread in natural products from terrestrial to marine organisms and irrespective of an isoprenic or polyketide origin, has been purported to possess enzyme-inhibiting functions.[40] Plants appear to use lactones to prevent being eaten and to avoid biofouling by bacteria while some animals use lactones to regulate their biochemical processes.[40] Since the butenolide **4** does not occur as an algal metabolite a question arises as to whether this unit has been biosynthesized by *A. dactylomela* as an additional ability (one more to add to acetylation and dehalogenation) to enhance the fitness of the sea hare.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer model 343 Plus polarimeter using a Na lamp at 20 °C. IR spectra were obtained with a Perkin–Elmer 1600/FTIR spectrometer. EIMS and HRMS spectra were taken on a Vg-Micromass Zab 2F spectrometer. ^{1}H NMR and ^{13}C NMR, HSQC, HMBC, COSY, and NOESY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125.7 MHz for ^{13}C NMR, using $CHCl_3$ as an internal standard. Two-dimensional spectra were obtained with the standard Bruker software. HPLC separations were performed with a Hewlett–Packard HP 1050 (Jaigel-Sil semipreparative column, 10 μ, 20×250 mm) with hexane–EtOAc mixture. The gel filtration column (Sephadex LH-20) used hexane–MeOH–$CHCl_3$ (3:1:1) as solvent. Merck Si gels 7734 and 7741 were used in column chromatography. The spray reagent for TLC was H_2SO_4–H_2O–AcOH (1:4:20).

3.2. Animal material

Eight specimens of *A. dactylomela* were collected off the SW coast of La Palma Island at −1.5 m depth. Specimens were dissected and their digestive system along with the mantle were separated and analyzed independently.

3.3. Extraction and isolation

A. dactylomela digestive glands were extracted with acetone at room temperature. The extract was concentrated to give a dark green residue (31.0 g) and partitioned with H_2O–CH_2Cl_2. The resulting fraction of CH_2Cl_2 (7.5 g) was then submitted to a gel filtration column to give fraction A (822.9 mg), which was subjected to flash chromatography on Si gel. The fraction eluted with hexane–EtOAc (8:2) gave a mixture that was further separated by HPLC (Jaigel-sil column 20×250 mm, flow 4.5 ml/min, hexane–EtOAc (8:2)) to yield the new sesquiterpene **1** (0.7 mg) and the known compound 8-acetylcaespitol (35.0 mg), caespitane (14.0 mg), and laucapyranoid A (4.0 mg). Fraction D was processed using flash chromatography on Si gel eluting with hexane–EtOAc (1:1) to give a mixture that was further separated by HPLC (Jaigel-sil column, flow 4.5 ml/min, hexane–EtOAc (1:1)) to yield the new compounds **2** (5.5 mg) and **4** (9.0 mg), and the known compound caespitenone (4.9 mg). Caespitol (73.6 mg) was isolated from fraction F by crystallization.

A. dactylomela mantles were extracted and processed following the same scheme. Thus, the acetonic extract was partitioned with H_2O–CH_2Cl_2 and the resulting fraction of CH_2Cl_2 (3.8 g) was submitted to a gel filtration column to give a fraction (308.3 mg), which after flash chromatography on Si gel eluted with hexane–EtOAc (1:1) gave a mixture that was further separated by HPLC (Jaigel-sil column, flow 4.5 ml/min, hexane–EtOAc (1:1)) to yield the new compounds **2** (30.0 mg), **3** (1.5 mg), and **4** (1.3 mg) together with the known metabolites caespitol (3.1 mg), 8-acetylcaespitol (3.3 mg), and furocaespitane (1.4 mg).

3.3.1. Compound 1. Colorless oil; $[\alpha]_D^{25}$ +492 (*c* 0.047, $CHCl_3$); IR ν_{max} (film) 3448, 1722 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 413/415/417/419 $[M-Me-H_2O]^+$ (<1, <1, <1, <1), 403/405/407/409 $[M-MeCO]^+$ (<1, <1, <1, <1), 385/387/389/391 $[M-MeCO-H_2O]^+$ (36, 87, 62, 11), 237/239/241 $[C_8H_{11}OBrCl]^+$ (30, 39, 3), 209/211/213 $[C_7H_{11}BrCl]^+$ (9, 12, 3), 173/175 $[C_7H_{10}Br]^+$ (13, 13); HREIMS 412.9480 (calcd for $C_{14}H_{20}O_2{}^{35}Cl^{79}Br_2$, 412.9518), 402.9596 (calcd for $C_{13}H_{22}O_2{}^{35}Cl^{79}Br_2$, 402.9675), 384.9512 (calcd for $C_{13}H_{20}O^{35}Cl^{79}Br_2$, 384.9569).

3.3.2. Compound 1a. ^{13}C NMR (50 MHz, $CDCl_3$) δ 210.9 (C-7), 81.5 (C-8), 71.1 (C-3), 62.1 (C-2), 59.3 (C-10), 58.2 (C-11), 44.8 (C-6), 42.3 (C-4), 35.7 (C-1), 35.5 (C-9), 31.1 (C-14), 24.8 (C-13), 23.9 (C-15), 23.0 (C-5), 19.1 (C-12).

3.3.3. Compound 2. White powder; $[\alpha]_D^{25}$ −1.72 (*c* 1.74, $CHCl_3$); IR ν_{max} (film) 3384 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* 316/318 $[M]^+$ (<1, <1), 298/300 $[M-H_2O]^+$ (2, 2), 236 $[M-HBr]^+$ (8), 23 (28), 139 (100); HREIMS 316.1050 (calcd for $C_{15}H_{25}O_2{}^{79}Br$, 316.1037), 298.0900 (calcd for $C_{15}H_{23}O^{79}Br$, 298.0932), 236.1751 (calcd for $C_{15}H_{24}O_2$, 236.1776).

3.3.4. Compound 3. Colorless oil; $[\alpha]_D^{25}$ −90.9 (*c* 0.03, $CHCl_3$); IR ν_{max} (film) 1738 cm^{-1}; ^{1}H and ^{13}C NMR, see

Table 1; EIMS *m/z* 234 $[M]^+$ (<1), 219 $[M-Me]^+$ (1), 140 (94), 125 (61), 96 (100); HREIMS 234.1665 (calcd for $C_{15}H_{22}O_2$, 234.1619), 219.1412 (calcd for $C_{14}H_{19}O_2$, 219.1385).

3.3.5. Compound 4. White powder; $[\alpha]_D^{25}$ +55 (*c* 0.25, $CHCl_3$); IR ν_{max} (film) 3352, 1751 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 322/324/326 $[M]^+$ (<1, <1, <1), 307/309/311 $[M-Me]^+$ (25, 33, 9), 243/245 $[M-Br]^+$ (20, 6), 225/227 $[M-Br-H_2O]^+$ (77, 26), 207 $[M-HBr-Cl]^+$ (100); HREIMS 321.9934 (calcd for $C_{12}H_{16}O_3{}^{35}Cl^{79}Br$, 321.9971), 306.9639 (calcd for $C_{11}H_{13}O_3{}^{35}Cl^{79}Br$, 306.9736), 243.0723 (calcd for $C_{12}H_{16}O_3{}^{35}Cl$, 243.1643), 225.0595 (calcd for $C_{12}H_{14}O_2{}^{35}Cl$, 225.0682), 207.0933 (calcd for $C_{12}H_{15}O_3$, 207.1021).

3.3.6. Compound 4a. ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 172.6 (C-10), 170.4 (C-8), 116.3 (C-9), 106.8 (C-7), 70.2 (C-3), 60.7 (C-2), 41.9 (C-4), 39.7 (C-1), 36.8 (C-6), 28.6 (C-5), 23.9 (2C, C-11, C-12).

3.4. Dehalogenation of caespitol

A solution of caespitol (20.0 mg) in DMF (1.5 ml) was treated with NaI–Zn and stirred at 115 °C for 2 h. The reaction was quenched with H_2O and extracted with EtOAc to give a mixture that after silica gel chromatography (hexane–EtOAc, 95:5) gave 7.9 mg of compound **2**.

3.5. Oxidation of compound 2

A solution of compound **2** (12.4 mg) in acetone was treated with Jones reagent and stirred at room temperature for 1.5 h. The reaction was quenched with H_2O and extracted with EtOAc to give 8.3 mg of compound **3**.

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