Biosynthetic Evidence Supporting the Generation of Terpene Chemodiversity in Marine Mollusks of the Genus Doriopsilla

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Pelseneeriol-1 and -2: new furanosesquiterpene alcohols from porostome nudibranch *Doriopsilla pelseneeri*

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Abstract—The paper reports the first chemical study of the porostome nudibranch *Doriopsilla pelseneeri* collected off the Portuguese coast (Atlantic Ocean). Two new furanosesquiterpene alcohols, pelseneeriol-1 (**1**) and pelseneeriol-2 (**2**), have been isolated together with known compounds, 15-acetoxy-*ent*-pallescensin-A (**5**), and dendocarbin-A (**6**), from the mantle of the nudibranch, whereas euryfuran (**3**) and drimane ester mixture **4** were identified in the extract of the internal glands. The structures of **1** and **2** have been determined by extensive spectroscopic studies as well as by comparison with literature model compounds. In order to assess the relative stereochemistry of **1** and **2**, full NMR assignment of related sponge metabolite microcionin-2 (**8**) and of co-occurring sesquiterpenes **9**–**11**, that have been re-isolated from the Mediterranean sponge *Fasciospongia cavernosa*, has been also conducted. In particular, the relative stereochemistry of tricyclic sesquiterpene microcionin-1 (**9**) has now been rigorously assigned by detailed analysis of NOE difference experiments.

1. Introduction

Nudibranchs are a group of shell-less opisthobranch molluscs, in which secondary metabolites play an important ecological role as defensive chemical weapons.[1–3] Most of these allomones are accumulated from dietary sources but some of them are biosynthesised de novo or obtained by bio-transformation of dietary metabolites.[4] The family Dendrodoridae is divided into the two genera *Dendrodoris* and *Doriopsilla* and comprises soft-bodied molluscs mostly devoid of mechanical protection, without radula. They suctorially feed exclusively on sponges and are chemically characterised by the presence of drimane sesquiterpenes.[1,2]

Few chemical studies have been published on the genus *Doriopsilla*,[5–7] including the recent reports on *Doriopsilla areolata* that showed the co-occurrence in this mollusc of two groups of sesquiterpenoids exhibiting drimane and *ent*-pallescensin A-like skeleton with opposite A/B ring junction.[6,7]

Further studies demonstrated that *D. areolata* is able to biosynthesise de novo both series of compounds.[8,9] Surprisingly, metabolites of both series were previously reported to co-occur in a sponge of genus *Dysidea*,[10] which was suggested to be potentially included in its diet.[8]

In this paper, we report the first chemical study on the related species *Doriopsilla pelseneeri* d'Oliveira 1895, that has resulted in the isolation of two new furanosesquiterpenes, pelseneeriol-1 (**1**) and pelseneeriol-2 (**2**), along with the known compounds **3**–**6**.

2. Results and discussion

D. pelseneeri is an endemic porostome nudibranch occurring in Iberian coastal waters (Atlantic and Mediterranean).[11] The mollusc (34 specimens) was collected in Arflor, Setúbal, off the Portuguese coast at a depth of 8–10 m, during May 2003, and immediately frozen at −20 °C. The mucus secreted by five individuals was also sampled and frozen. The biological material was subsequently transferred to ICB in Italy for chemical analysis. Frozen specimens were carefully dissected in mantle and inner organs, which were separately extracted by acetone exhaustively under ultrasound vibration. The mucous secretion was directly extracted by diethyl ether. The

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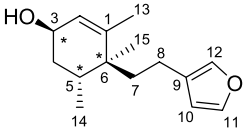
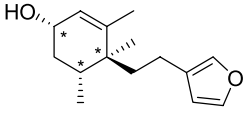
ethereal soluble portions of acetone extracts of both mantle and internal glands were analysed along with mucus extract by TLC chromatography displaying different secondary metabolite patterns. In particular, the extract of the internal

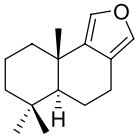


*relative stereochemistry

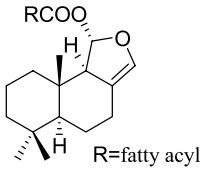
1 pelseneeriol-1



2 pelseneeriol-2

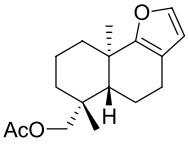


3 euryfuran

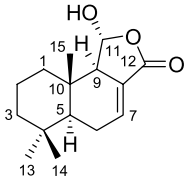


R=fatty acyl

4 drimane esters



5 15-acetoxy-*ent*-pallescensin-A



6 dendocarbin-A

part (539 mg) was characterized by the presence of a main Ehrlich positive spot at R_f 0.5 (light petroleum ether/diethyl ether, 95:5) along with usual lipids and sterols and a minor non-polar compound at R_f 0.5 (light petroleum ether), whereas the mantle and mucus extracts were found to contain a series of metabolites at R_f 0.5 (light petroleum ether/diethyl ether, 9:1) and at R_f 0.40–0.25 (light petroleum ether/diethyl ether, 1:1).

An aliquot (61 mg) of the internal gland extract was purified on a silica-gel column (light petroleum ether/diethyl ether gradient) to give, in order of increasing polarity, euryfuran (**3**, 2.0 mg) and drimane esters mixture (**4**, 9.1 mg).

The mantle extract (196 mg) was chromatographed on a Sephadex LH-20 column using MeOH/$CHCl_3$ 1:1 as eluent. The fraction containing Ehrlich positive spot at R_f 0.5 (light petroleum ether/diethyl ether, 9:1) (8.7 mg) was further purified by a silica-gel column (light petroleum ether/diethyl ether gradient) to give 15-acetoxy-*ent*-pallescensin-A (**5**, 1.0 mg). Fractions containing spots at R_f 0.40–0.25 (light petroleum ether/diethyl ether, 1:1) were submitted to *n*-phase HPLC (*n*-hexane/EtOAc, 85:15) to obtain two unprecedented compounds, **1** (0.8 mg) and **2** (0.7 mg), and the known dendocarbin-A (**6**, 0.5 mg), in order of increasing retention time.

The mucus extract (5.9 mg) was purified by Sephadex LH-20 chromatography in the same manner as mantle extract, to obtain compound **5** (1.0 mg), and a mixture (0.9 mg) which was analysed by HPLC resulting to be constituted by compounds **1**, **2** and **6**.

The known compounds, drimane ester mixture **4** and its work-up derivative euryfuran (**3**), isolated from the internal glands, 15-acetoxy-*ent*-pallescensin-A (**5**) and dendocarbin-A (**6**), isolated from both mantle and mucus, were identified by comparison of spectral data with those reported in the literature. Drimane ester mixture **4** has been reported to occur in several dendrodorid nudibranch species,[1,2] whereas 15-acetoxy-*ent*-pallescensin-A (**5**) has been previously isolated only from the mantle of different collections of *D. areolata*[5–7] and dendocarbin-A (**6**) has been recently found in *Dendrodoris carbunculosa*.[12]

The structure of compound **6** was previously reported[12] except for the relative stereochemistry at C-11, due to the difficulty of detecting both H-11 and C-11 signals in NMR spectra either in CD_3OD or in C_6D_6. We were able to perform NOE difference experiments in $CDCl_3$ on dendocarbin-A (**6**) that led to the assignment of the relative configuration at C-11 as reported. In fact, in addition to expected NOE interactions between H-5 (δ 1.36) and H-9 (δ 2.48), a diagnostic NOE effect was observed between H-11 (δ 5.64) and H_3-15 (δ 0.85). However, the assigned stereochemistry matches that established for the acetyl derivative of dendocarbin-A.[12]

The structures of the novel sesquiterpene alcohols **1** and **2**, that we named pelseneeriol-1 and pelseneeriol-2, respectively, were determined as follows. HRESIMS analysis indicated that compounds **1** and **2** were isomers with the same molecular formula $C_{15}H_{22}O_2$. Comparison of their ^{1}H NMR spectra (Table 1) clearly suggested a close relationship between the two molecules, both exhibiting a terminal β-substituted furan moiety [δ 7.34 (H-11), 7.21 (H-12) and 6.26 (H-10) in **1**; δ 7.34 (H-11), 7.20 (H-12) and 6.25 (H-10) in **2**], a trisubstituted double bond [δ 5.64 (H-2) in **1**; δ 5.49 (H-2) in **2**], and a secondary hydroxyl function [δ 4.08 (H-3) in **1**; δ 4.23 (H-3) in **2**]. The presence of three methyl signals [a singlet (H_3-15) at δ 0.85 in **1**, 0.92 in **2**; a doublet (H_3-14) at δ 0.92 in **1**, 0.91 in **2**; a broad singlet (H_3-13) at δ 1.70 in **1**, 1.68 in **2**] in the ^{1}H NMR spectra of both molecules was consistent with sesquiterpene structures, that were further supported by ^{13}C NMR data (Table 1). Analysis of 2D NMR (^{1}H–^{1}H COSY, HSQC and HMBC) spectra of **1** and **2** suggested for both compounds a rearranged $\Delta^{1,6}$ monocyclofarnesol skeleton, exhibiting an hydroxyl group at C-3. In fact, diagnostic ^{1}H–^{1}H COSY correlations were observed between the carbinolic proton H-3 (δ 4.08 in **1**; δ 4.23 in **2**) and either the olefinic proton H-2 (δ 5.64 in **1**; δ 5.49 in **2**) or the methylene H_2-4 (δ 1.62–1.64 in **1**; δ 1.39–1.82 in **2**), which was further coupled to the methine H-5 (δ 2.09 in **1**; δ 1.85 in **2**). This structural hypothesis was further supported by comparison of ^{1}H NMR values of **1** and **2** with those of literature sesterterpenes containing the same cyclic

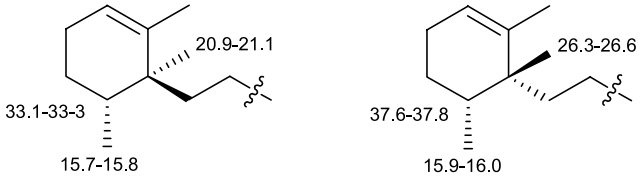
Table 1. NMR data[a] of pelseneeriol-1 (**1**), pelseneeriol-2 (**2**), fulvanin-1 (**7**) and microcionin-2 (**8**)

	1				**2**				**7**	**8**
Position	δ ^{13}C[b]	δ ^{1}H[c]	m, J (Hz)	HMBC[d]	δ ^{13}C[b]	δ ^{1}H[c]	m, J (Hz)	HMBC[d]	δ ^{13}C[e]	δ ^{13}C[e]
1	145.4	—	—	H_3-13, H_3-15	142.9	—	—	H_3-13, H_3-15	139.0	139.1
2	126.0	5.64	br d, 5	H_3-13	129.2	5.49	br s	H_3-13	124.7	123.0
3	64.4	4.08	br s	—	67.9	4.23	m ($w_{1/2}$=20)	—	25.5	24.0
4	35.9	1.62	m	H_3-14	37.3	1.39	ddd (H_{ax}), 12,12,10	H_3-14	27.0	27.5
		1.64	m			1.82	m (H_{eq})			
5	27.6	2.09	m	H_3-14, H_3-15	31.6	1.85	m	H_3-14, H_3-15	33.3	37.7
6	41.0	—	—	H_3-13, H_3-14, H_3-15	41.0	—	—	H_3-13, H_3-14, H_3-15	40.5	39.6
7	36.2	1.68	m	H_3-15	36.0	1.63	m	H_3-15	34.4	36.3
8	19.6	2.04	m	—	19.5	2.02	ddd, 6,12,15	—	35.8	20.9
		2.41	ddd, 6, 9, 14			2.34	ddd, 4,11,15			
9	125.4	—	—	—	125.4	—	—	H-11, H-12	161.6	126.1
10	110.9	6.26	br s	—	110.9	6.25	br s	—	114.7	110.9
11	142.8	7.34	br s	—	142.8	7.34	br s	H-10, H-12	167.3	142.6
12	138.4	7.21	br s	—	138.4	7.20	br s	H-10, H-11	19.1	138.4
13	19.1	1.70	br s	—	18.9	1.68	br s	—	19.1	19.7
14	15.6	0.92	d, 7	—	15.9	0.91	d, 7	—	15.8	16.0
15	19.5	0.85	s	—	20.6	0.92	s	—	21.0	26.3

[a] Bruker DPX 500 and AVANCE 400 MHz spectrometers, $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$ (δ 7.26) and to $CDCl_3$ (δ 77.0).
[b] By DEPT, HSQC and HMBC (J=10 Hz) experiments.
[c] By ^{1}H–^{1}H COSY and HSQC experiments.
[d] Significant HMBC correlations (J=10 Hz).
[e] Assignments from Refs. 14 and 15.

moiety.[13] All proton and carbon assignments of pelseneeriols are reported in Table 1.

Comparison of proton spectra and in particular of the carbinolic signal multiplicity indicated that the structures of the two compounds could differ only in the relative stereochemistry of the hydroxyl group, which was suggested to be axially oriented in pelseneeriol-1 (**1**) (H-3$_{eq}$ resonates at δ 4.08 as a broad singlet), and equatorial in pelseneeriol-2 (**2**) [H-3$_{ax}$ resonates at δ 4.23 as a multiplet ($w_{1/2}$=20 Hz)]. Analysis of carbon values of the cyclohexene ring of both compounds further supported this suggestion. In fact, the different shift values at C-3, C-4, and C-5 due to axial or equatorial hydroxyl substituent were in agreement with the expected calculated effects.[14] In addition, for pelseneeriol-2 (**2**), a diagnostic positive NOE effect was observed between H-3 (δ 4.23) and H-5 (δ 1.85 m), thus inferring that both H-5 and H-3 were axially oriented.



a **b**

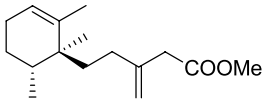
The relative stereochemistry of the methyl groups at C-5 and C-6 was suggested to be *cis* in both sesquiterpenes by comparison of the ^{13}C chemical shift of Me-14 (δ 15.6 in **1** and δ 15.9 in **2**) and Me-15 (δ 19.5 in **1** and δ 20.6 in **2**) with carbon values of natural terpenes containing *cis* (**a**) or *trans* (**b**) substructure. The ^{13}C chemical shift of Me-14 has similar value (15.7–16.0 ppm) in *cis*[15,16] and *trans*[17–19] isomers whereas the carbon value of Me-15 is smaller in *cis* (20.9–21.1 ppm) than in *trans* (26.3–26.6 ppm) isomer due to the greater γ-type interactions between the two methyl groups in *cis* compounds.

In particular, two model sponge sesquiterpenes exhibiting **a** or **b** substructure, (+)-5*R*,6*S*-fulvanin-1 (**7**)[15,16,20] and (−)-5*R*,6*R*-microcionin-2 (**8**),[18,21,22] the stereochemistry of which have been secured by stereospecific synthesis,[18,20] were considered (see Table 1). The close similarity of carbon data of pelseneeriols with those of fulvanin-1 clearly indicated the same relative *cis*-stereochemistry of the methyl groups at C-5 and C-6. However, unfortunately, the absolute stereochemistry of compounds **1** and **2** was not determined due to the instability of both molecules that slowly degraded in chloroform solution.

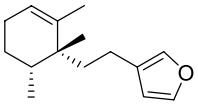
Metabolites related to pelseneeriols, the above cited microcionin-2 (**8**), along with microcionin-1 (**9**), microcionin-3 (**10**) and microcionin-4 (**11**), were found in the Mediterranean sponge *Fasciospongia cavernosa* (incorrectly reported in the first paper as *Microciona toxystila*),[21] which could potentially be a prey of the nudibranch. In fact, even though microcionin-2 (**8**) and microcionin-4 (**11**) exhibit the methyl groups at C-5 and C-6 *trans*-oriented, the co-occurring microcionin-3 (**10**) could be a possible precursor of both series of compounds: microcionins in *F. cavernosa* and pelseneeriols in *D. pelseneeri*.

In the course of this study, microcionins 1–4 have been re-isolated from a new collection of the sponge *F. cavernosa* and fully characterised by extensive NMR analysis. Proton and carbon assignments of compounds **8–11** are reported in the Section 4. The relative stereochemistry of microcionin-4 (**11**), which is closely related to microcionin-2 (**8**), was suggested by biogenetic considerations whereas the relative stereochemistry of microcionin-1 (**9**), which surprisingly exhibits H$_3$-14 and H$_3$-15 *cis*-oriented, was established by detailed analysis of 1D and 2D NMR experiments and in particular of NOE difference spectra. In fact, a diagnostic

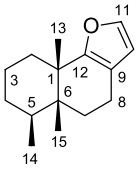
NOE effect observed between H_3-15 (δ 0.85) and H_3-13 (δ 1.08) inferred the 1,6-*cis*-junction of the carbon skeleton of **9**. Furthermore, irradiation of H_3-15 (δ 0.85) induced significant enhancements on H_2-2_{ax} (δ 1.51) and H_2-4_{ax} (δ 1.22), implying the axial orientation of H_3-15 and, consequently, the equatorial orientation of H_3-13. Finally, analysis of the coupling constants of H-5 (δ 1.68), that were calculated as $J_{H5ax-H4ax}=12$ Hz, $J_{H5ax-H4eq}=4$ Hz by decoupling of geminal methyl H_3-14 (δ 0.83), indicated that H_3-14 was equatorial, *cis*-oriented with respect to H_3-15. Accordingly, irradiation of H-5 only resulted in a weak enhancement of the multiplet at δ 2.34 (H_2-8).



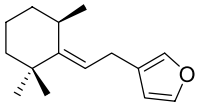
7 (+)-5*R*,6*S*-fulvanin-1



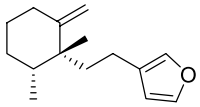
8 (-)-5*R*,6*R*-microcionin-2



9 microcionin-1*



10 microcionin-3*



11 microcionin-4*

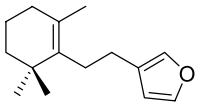
*relative stereochemistry

It is interesting to observe that, among microcionins, the methyls at C-5 and C-6 are *cis*-oriented only in microcionin-1 (**9**). This relative stereochemistry is analogous to that observed in pelseneeriols.

3. Conclusions

Analogously with other *Doriopsilla* species,[5–7] *D. pelseneeri* has been found to contain sesquiterpenes structurally related to typical metabolites of sponges of genus *Dysidea* that could be included in the diet of the nudibranch. So, a dietary origin could be reasonably suggested for compounds **1–6**, even though no direct observation supports this hypothesis. However, recent biosynthetic studies on sesquiterpene metabolites have been rigorously conducted on Atlantic porostome nudibranch *D. areolata*.[8,9] Very interestingly, this mollusc was proved to be able to produce de novo molecules of both *ent*-pallescensin-like and drimane skeleton (e.g., **5** and **6**) which could also characterise an organism included in its diet. In fact, compounds with both carbon skeletons were found to co-occur in an Australian *Dysidea* sponge.[10]

The de novo biosynthesis could also be suggested for all the sesquiterpenes present in *D. pelseneeri*. Preliminary biosynthetic studies on this nudibranch have shown significant incorporation of labelled piruvate into drimane ester mixture (**4**) (Fontana, personal communication). Therefore, *D. pelseneeri* seems to be able to produce its drimane sesquiterpene metabolites as other porostome nudibranchs, whereas the biosynthesis of pelseneeriols and *ent*-pallescensin derived compounds is strongly suspected but remains to be rigorously proved. Butler and Capon suggested that a common hypothetical intermediate **12** could lead to sesquiterpenes of both series, *ent*-pallescensin and drimane, in Australian sponge *Dysidea* sp.[10] Analogously, the isomer of **12**, microcionin-3 (**10**) could be considered an hypothetical intermediate for the three different sesquiterpenes skeletons found in *D. pelseneeri.*



12

4. Experimental

4.1. General experimental procedures

Silica-gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. HPLC purification was carried out on a Waters liquid chromatograph equipped with a Waters R401 RI detector. Optical rotations were measured on a Jasco DIP 370 digital polarimeter.

NMR experiments were recorded at ICB NMR Service. 1D and 2D NMR spectra were acquired in $CDCl_3$ (δ values are reported referred to C*H*Cl_3 at 7.26 ppm) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the *Z*-axis, and on a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the *Z*-axis. ^{13}C NMR were recorded on a Bruker DPX-300 operating at 300 MHz (δ values are reported to C*D*Cl_3, 77.0 ppm) using a dual probe.

EIMS were determined at 70 eV on a HP-GC 5890 series II mass spectometer. High resolution ESIMS were performed on a Micromass Q-TOF Micro™ coupled with a HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation <5 ppm RMS in the presence of a known lock mass).

4.2. Biological material

Thirty-four specimens of *D. pelseneeri* (average size 2.5 cm) were collected off Arflor (38°30′24″N; 08°55′09″W), Setúbal, along the Western coast of Portugal, in May 2003, at a depth of 8–10 m. The mollusc was immediately frozen, transferred to ICB, and stored at -20 °C till the extraction. The taxonomic identification of *D. pelseneeri* has been made by one of us (G. Calado). A voucher specimen (preserved in absolute ethanol) is deposited at 'Instituto Português de Malacologia', Portugal, reference number IPM.MO.100.

A sample of *F. cavernosa* was collected at Torre Annunziata, Naples, in January 2005, at a depth of 1 m. A voucher specimen is at ICB (FCav-1).

4.3. Isolation procedure

Frozen *D. pelseneeri* (34 individuals, dry weight 10 g) were dissected into mantle and inner organs. Each part was separately extracted with acetone (3×150 mL, 2 min in ultrasonic bath). Each acetone extract was evaporated under vacuum and the resulting aqueous phases were extracted with Et_2O (3×30 mL). After evaporation of the solvent the organic layers gave crude extracts: 196 mg from the mantle and 539 mg from the inner organs. The mucus was directly extracted by diethyl ether (3×10 mL) to obtain 5.9 mg of a crude extract. A sample of *F. cavernosa* was immersed in acetone (50 mL) and extracted by using ultrasonic vibrations for 2 min. The treatment was repeated three times. The acetone extracts were combined and concentrated, then the aqueous residual was partitioned with diethyl ether (3×30 mL). After removing the solvent, the organic phase gave 957 mg of a crude residue.

An aliquot (61 mg) of the extract of internal glands of the nudibranch was chromatographed on a silica-gel column packed with light petroleum ether and eluted with light petroleum ether with increasing amounts of diethyl ether. Fractions eluted with light petroleum ether and light petroleum ether/diethyl ether, 95:5, were concentrated to give compound **3** (2.0 mg) and drimane ester mixture **4** (9.1 mg), respectively. The mantle extract (196 mg) was chromatographed on a Sephadex LH-20 column using MeOH/$CHCl_3$ 1:1 as eluent. The fraction (8.7 mg) containing the Ehrlich positive spot less polar than sterols was submitted to a pipette-pasteur silica-gel column to obtain compound **5** (1.0 mg). The fraction (6.2 mg) containing spots at R_f 0.40–0.25 was further purified by HPLC [column Phenomenex-Kromasil (5 μm, 100 Å, 250×4.60 mm); *n*-hexane/EtOAc 85:15 (flow 1 mL/min)] to yield **1** (0.8 mg), **2** (0.7 mg) and **6** (0.5 mg), in order of increasing retention time.

The mucus ether extract (5.9 mg) was chromatographed by Sephadex LH-20 column eluted with MeOH/$CHCl_3$ 1:1, to obtain compound **5** (1.0 mg) and a mixture (0.9 mg) which was analysed by HPLC [column Phenomenex-Kromasil (5 μm, 100 Å, 250×4.60 mm); *n*-hexane/EtOAc 85:15 (flow 1 mL/min)] Compounds **1**, **2** and **6** were present in the same ratio as the mantle extract.

An aliquot (300 mg) of the extract of *F. cavernosa* was submitted to a silica-gel column (light petroleum ether/diethyl ether gradient). All fractions containing microcionins were eluted by light petroleum ether. The fraction (10 mg) containing less polar metabolite was submitted to a pipette-pasteur $AgNO_3$–SiO_2 column (light petroleum ether/benzene, 95:5) to obtain pure compound **9** (1.5 mg). The other fractions (130 mg) were combined and purified on a $AgNO_3$–SiO_2 column (light petroleum ether/benzene gradient) to give compounds **8** (22.1 mg), **10** (22.7 mg), and **11** (5.5 mg).

Compounds **3**, **4** and **5** were identified by comparing their spectral data with those of standard samples.

4.3.1. Compound 1. Colourless oil; $[\alpha]_D$ +9.4 (*c* 0.04, $CHCl_3$); ^1H and ^{13}C NMR (Table 1); HRESIMS (positive) *m/z* 257.1521 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_2Na$ 257.1517).

4.3.2. Compound 2. Colourless oil; $[\alpha]_D$ −89.9 (*c* 0.03, $CHCl_3$); ^1H and ^{13}C NMR (Table 1); HRESIMS (positive) *m/z* 257.1507 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_2Na$ 257.1517).

4.3.3. Compound 6. Colourless oil; $[\alpha]_D$ −27.3 (*c* 0.1, $CHCl_3$), $[\alpha]_D$ lit.[12] −10 (*c* 0.14, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (1H, dd, J=3.3, 6.9 Hz, H-7), 5.64 (1H, br d, J=5.6 Hz, H-11), 3.61 (br s, OH), 2.48 (1H, m, H-9), 2.41 (1H, m, H_2-6a), 2.07 (1H, m, H_2-6b), 1.81 (1H, m, H_2-1a), 1.52 (2H, m, H_2-2), 1.51 (1H, m, H_2-3a), 1.36 (1H, dd, J=5.3, 11.6 Hz, H-5), 1.31 (1H, m, H_2-1b), 1.26 (1H, m, H_2-3b), 0.94 (3H, s, H_3-14), 0.92 (3H, s, H_3-13), 0.85 (3H, s, H_3-15); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.5 (C-12), 136.6 (C-7), 127.9 (C-8), 98.3 (C-11), 59.1 (C-9), 49.3 (C-5), 42.1 (C-3), 39.2 (C-1), 33.8 (C-10), 33.1 (C-13), 32.9 (C-4), 24.9 (C-6), 21.3 (C-14), 18.2 (C-2), 14.6 (C-15); ^{13}C NMR (75 MHz, CD_3OD) δ 170.3 (C-12), 137.7 (C-7), 129.9 (C-8), 60.5 (C-9), 50.6 (C-5), 43.3 (C-3), 40.5 (C-1), 35.4 (C-10), 33.6 (C-13), 33.6 (C-4), 25.8 (C-6), 21.8 (C-14), 19.3 (C-2), 14.8 (C-15); HRESIMS (positive) *m/z* 259.1660 $[M+Na]^+$ (calcd for $C_{15}H_{24}O_2Na$ 259.1674).

4.3.4. Compound 8. Colourless oil; $[\alpha]_D$ −26.4 (*c* 1.4, $CHCl_3$), $[\alpha]_D$ lit.[21] −58.3; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (1H, br s, H-11), 7.21 (1H, br s, H-12), 6.27 (1H, br s, H-10), 5.43 (1H, m, H-2), 2.42 (1H, ddd, J=5.5, 13.5, 13.5 Hz, H_2-8a), 2.32 (1H, ddd, J=3.9, 13.5, 13.5 Hz, H_2-8b), 1.98 (2H, m, H_2-3), 1.68 (1H, m, H_2-7a), 1.66 (3H, br s, H_3-13), 1.63 (2H, m, H_2-4a and H-5), 1.53 (1H, m, H_2-7b), 1.47 (1H, m, H_2-4b), 1.08 (3H, s, H_3-15), 0.99d (3H, d, J=6.8 Hz, H_3-14); ^{13}C NMR (Table 1); EIMS *m/z* (%) 218 (M^+, 10), 203 (8), 123 (100), 109 (41), 95 (53), 81 (71).

4.3.5. Compound 9. Colourless oil; $[\alpha]_D$ +47.6 (*c* 0.06, $CHCl_3$), $[\alpha]_D$ lit.[21] +7; ^1H NMR (600 MHz, $CDCl_3$); δ 7.25 (1H, d, J=1.7 Hz, H-11), 6.14 (1H, d, J=1.7 Hz, H-10), 2.34 (2H, m, H_2-8), 2.12 (1H, br d, J=14 Hz, H_2-2eq), 1.71 (1H, m, H_2-7a), 1.68 (1H, m, H-5), 1.58 (1H, m, H_2-7b), 1.51 (1H, m, H_2-2ax), 1.50 (1H, m, H_2-3ax), 1.28 (1H, m, H_2-4eq), 1.22 (1H, ddd, J=13.2, 12.9, 3.2 Hz, H_2-4ax), 1.08 (3H, s, H_3-13), 1.00 (1H, dt, J=13.1, 3.9 Hz, H_2-3eq), 0.85 (3H, s, H_3-15), 0.83 (3H, d, J=6.9 Hz, H_3-14); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.7 (C-12), 140.2 (C-11), 115.1 (C-9), 110.1 (C-10), 40.2 (C-1), 39.1 (C-6), 32.3 (C-2), 31.9 (C-5), 30.7 (C-4), 29.3 (C-7), 25.9 (C-13), 23.7 (C-3), 18.6 (C-8), 16.8 (C-14), 14.7 (C-15); EIMS *m/z* (%) 218 (M^+, 32), 203 (100), 147 (20), 133 (28), 109 (20), 91 (18).

4.3.6. Compound 10. Colourless oil; $[\alpha]_D$ +24.4 (*c* 1.70, $CHCl_3$), $[\alpha]_D$ lit.[21] +36.5; ^1H NMR (600 MHz, $CDCl_3$) δ 7.35 (1H, br s, H-11), 7.20 (1H, br s, H-12), 6.27 (1H, br s, H-10), 5.39 (1H, t, J=7.1 Hz, H-7), 3.21 (1H, dd, J=7.2, 16.4 Hz, H_2-8a), 3.11 (1H, dd, J=7.0, 16.4 Hz, H_2-8b), 2.96 (1H, m, H-1), 1.77 (1H, m, H_2-3a), 1.59 (1H, m, H_2-2a), 1.52 m (1H, m, H_2-2b), 1.45 (1H, m, H_2-4a), 1.43 (1H, m, H_2-3b), 1.29 (1H, m, H_2-4b), 1.15 (3H, d, J=7.6 Hz, H_3-13), 1.11 (3H, s, H_3-14 or H_3-15), 1.08 (3H, s, H_3-15 or H_3-14); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.4 (C-6), 142.7 (C-11), 138.8 (C-12), 124.9 (C-9), 119.0 (C-7), 111.1

(C-10), 41.4 (C-4), 36.2 (C-5), 32.8 (C-2), 31.6 (C-15 or C-14), 30.5 (C-14 or C-15), 29.7 (C-1), 22.9 (C-8), 21.5 (C-13), 17.7 (C-3); EIMS *m/z* (%) 218 (M^+, 60), 175 (10), 147 (42), 109 (75), 95 (53), 81 (100).

4.3.7. Compound 11. Colourless oil; $[\alpha]_D$ +131.2 (*c* 0.05, $CHCl_3$), $[\alpha]_D$ lit.[21] +98.3; ^1H NMR (400 MHz, $CDCl_3$); δ 7.34 (1H, br s, H-11), 7.20 (1H, br s, H-12), 6.26 (1H, br s, H-10), 4.78 (1H, br s, H_2-13a), 4.66 (1H, br s, H_2-13b), 2.19 (2H, m, H_2-8a, H_2-2a), 2.08 (2H, m, H_2-8b, H_2-2b), 1.82 (1H, m, H_2-3a), 1.76 (1H, m, H_2-3b), 1.46 (2H, m, H_2-4), 1.41 (1H, m, H-5), 1.40 (2H, m, H_2-7), 0.89 (3H, d, J= 6.3 Hz, H_3-14), 1.12 (3H, s, H_2-15); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.5 (C-1), 142.6 (C-11), 138.5 (C-12), 125.9 (C-9), 111.1 (C-10), 107.7 (C-13), 43.1 (C-4), 42.0 (C-6), 33.5 (C-2), 31.6 (C-5), 30.6 (C-7), 27.9 (C-3), 22.6 (C-15), 19.2 (C-8), 16.1 (C-14); EIMS *m/z* (%) 218 (M^+, 18), 124 (35), 109 (100), 95 (45), 81 (76).

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