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New oxygenated diterpenes from an Australian nudibranch of the genus *Chromodoris*

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ABSTRACT

Chemical analysis of a chromodorid nudibranch has provided two new diterpene metabolites **1** and **2** together with the known metabolites **3**–**6**. In an NMR study, the dialdehyde metabolite **1** underwent facile conversion to cyclic hemiacetals **8**–**9** on exposure to methanol, a reaction that mimics chemical conversions that may occur during the isolation of some diterpenes from molluscs and sponges. Compounds **1**, **2**, **5** and **8** showed moderate cytotoxicity against P388 cells (IC_{50}=1.2–4.1 μg/mL).

1. Introduction

Reported biological activities that include cytotoxic and antiviral activities have led to the discovery in marine sponges of a wealth of highly oxygenated diterpene metabolites possessing either the spongian or the isocopalane skeletons,[1] and to increasing interest in the synthesis of these compounds.[2] Certain groups of molluscs, predominantly those of the order (family) Chromodorididae, feed on these diterpene-containing sponges and store the metabolites in specialised anatomical compartments known as mantle dermal formations (MDFs) within their mantle tissue.[3] Examples of oxygenated diterpenes are the aplysillin metabolites (*Chromodoris luteorosea*[4] and *Chromodoris geminus*[5]), the aplyroseols and related spongian compounds from *Chromodoris tasmaniensis*,[6] *Chromodoris inopinata*,[5] *Chromodoris obsoleta*,[7] and *Chromodoris epicuria*[8] (syn. *Ceratosoma brevicaudatum*).[3] Diterpene chemistry is also shown by *Chromodoris hamiltoni*, for which analysis of two different populations has yielded the hamiltonins, with a 3-homo-4,5-seco-spongian skeleton, and two new spongian diterpenes.[9] Specimens of the mollusc *Glossodoris atromarginata* collected from Sri Lanka[10] and from Australia[11] show oxygenated spongian diterpene chemistry as does a Red Sea population of *Glossodoris cincta*.[12] In contrast, populations of *Glossodoris averni* collected from diverse Indo-Pacific locations show a different chemical pattern and usually sequester sesterterpene-derived scalarane metabolites from dictyoceratid sponges, including those of the genus *Cacospongia*.[13] This mollusc can selectively modify the sponge products by oxidation yielding 12-keto products that have not so far been encountered in the parent sponges. These biotransformations may enhance the in situ biological effects of these products in *G. averni* or serve to detoxify the ingested compounds.[14] Finally *G. atromarginata* from India, in contrast to other Indo-Pacific populations, also contains scalarane metabolites.[15]

We now report the isolation of two new diterpenes (**1** and **2**) together with the four known diterpenes (**3**–**6**)[7,16–18] from a single specimen of a chromodorid mollusc. A full taxonomic identification could not be undertaken owing to the damaged condition of the specimen, but preliminary assignment suggested that the sample was a *Chromodoris* sp. The anatomical distribution of the isolated diterpene compounds within the various tissue types of the mollusc was also explored. During the chemical investigation, it was found that the dialdehyde diterpene **1** underwent facile ring closure to acetal-based products in the presence of certain solvents. The presence of methanol during a separation step led to isolation of hemiacetal **7** while brief exposure of **1** to methanol-d_4 led to rapid conversion to cyclic products such as **8** and **9**, chemistry; this chemistry is highly relevant to some of the existing literature on sponge and nudibranch diterpenes.[7,16]

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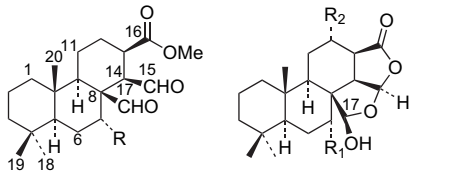
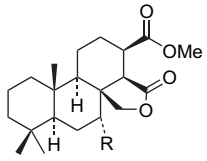
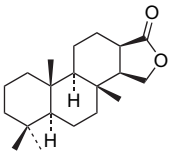


1 R = OAc
10 R = H
11 R = OH

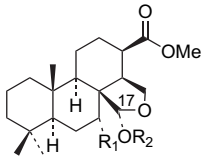
2 R_1 = H, R_2 = OAc
5 R_1 = OAc, R_2 = H
12 R_1 = H, R_2 = H
13 R_1 = $OCOC_3H_7$, R_2 = H



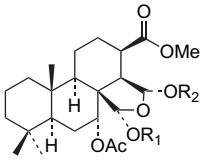
3 R = H
4 R = OAc



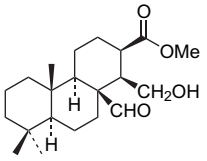
6



7 R_1 = H, R_2 = CH_3
14 R_1 = OAc, R_2 = CH_3
16 R_1 = H, R_2 = H
17 R_1 = H, R_2 = CD_3



8 R_1 = H, R_2 = CD_3
9 R_1 = CD_3, R_2 = H



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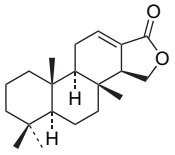

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2. Results and discussion

A single large specimen of *Chromodoris* sp. was collected by SCUBA from the Gneerings Reef offshore from Mooloolaba in South East Queensland. The nudibranch was dissected into two different anatomical components, namely the mantle tissue and the internal organs. Extraction of the mantle tissue with acetone gave a terpene-rich organic extract that was fractionated by silica flash chromatography (hexanes/EtOAc), followed by normal phase HPLC using hexanes/EtOAc. Using these separation protocols, two new (**1** and **2**) and four known metabolites were obtained. The known metabolites were identified by comparison of NMR data with the literature; these were dendrillol-3 (**3**),[17] 7α-acetoxydendrillol-3 (**4**),[7] 7α-acetoxy-17β-hydroxy-15,17-oxidospongian-16-one (**5**)[16] and spongian-16-one (**6**).[18] The NMR values for some protons of the known compounds **3** and **5** were not listed in the previous literature, and we now provide the full NMR assignment based on 2D NMR experiments (Table 1). This is also the first report of ^{13}C NMR assignments for compound **5**.

The new compound **1** was isolated as a colourless oil, and had a molecular formula of $C_{23}H_{34}O_6$ inferred from HRESIMS (*m*/*z* 429.2254 $[M+Na]^+$). The ^1H NMR spectrum showed the presence of three methyl singlets (δ_H 0.74, 0.75, 0.76), one acetoxymethyl (δ_H 2.05), one carboxymethyl (δ_H 3.65) and two aldehyde protons (δ_H 9.68, 9.97) (Table 1). Except for the two aldehyde protons, these signals were similar to those of 7α-acetoxydendrillol-3 (**4**), which was reported from the nudibranch *C. obsoleta*.[7] Other ^1H NMR resonances were also comparable to those of compound **4**, except for the absence of the distinctive AB system for H_2-17 (J=10 Hz) that belongs to the γ-lactone ring. The ^{13}C NMR spectrum also confirmed the absence of γ-lactone signals and that these had been replaced by two aldehyde carbons. The aldehyde groups (δ_H 9.68; δ_C 200.0 and δ_H 9.97; δ_C 202.4) were assigned to C-15 and C-17, respectively, based on HMBC correlations and a small vicinal coupling (J=0.7 Hz) between δ_H 9.68 (H-15) and 2.67 (H-14). HMBC correlations from both δ_H 5.86 (H-7, t, J=3.0 Hz) and 2.05 ($OCOCH_3$, s) to δ_C 169.3 confirmed the position of the acetoxy group at C-7. The small coupling constant of 3.0 Hz between H-7 and H_2-6 indicated the equatorial position of H-7 and hence an α-orientation of the acetoxyl. As expected, the ester carbon at δ_C 173.5 (C-16) showed HMBC correlations from δ_H 1.60 (H-12α), 2.46 (H-12β), 3.25 (H-13) and 3.65 (OMe). The relative configuration of compound **1** was deduced from a 2D NOESY experiment. Key NOE correlations from 15-CHO to H-7 and H-14, and from 17-CHO to H-6β, H-7 and CH_3-20 indicated that both aldehyde groups were β-orientated (Fig. 1). The cyclohexane ring C was in a chair conformation based on the NOEs observed between H-14/H-9 and H-14/H-12α. Furthermore, NOESY cross-peaks from H-13 (J=5.5, 1.9 Hz) to H-12α, H-12β and H-14 confirmed the α-orientation of H-13. Therefore, the new compound **1** was assigned as methyl 7α-acetoxy-8β,14β-diformylpodocarpane-13β-carboxylate. Two closely related compounds, **10** and the 7-hydroxyl functionalised **11**, have been synthesised as key intermediates in the formation of various aplyroseol-type diterpenes.[19,20]

The new compound **2** displayed a sodiated molecular ion peak in the HRESIMS at *m*/*z* 415.2093, corresponding to a molecular formula $C_{22}H_{32}O_6$. The ^1H and ^{13}C NMR spectra presented three methyls (δ_H 0.81, 0.85, 0.88), an acetoxymethyl (δ_H 2.06) and two acetal protons (δ_H 5.49 and 6.09), which were similar to those of a known compound **5** previously isolated from the sponge *Igernella notabilis*.[16] HMBC and 1D TOCSY experiments revealed that the acetoxy group was attached to C-12, as opposed to C-7 in compound **5**. In the 1D TOCSY spectra, irradiation of H-5 (δ_H 0.98) resulted in the enhancement of H-6α, H-6β, H-7α and H-7β, while irradiation of H-15 (δ_H 6.09) resulted in the enhancement of H-12, H-13 and H-14. The relative configuration of 17-OH was assigned as β, based on NOEs observed from H-17 to H-6β, H-7β and CH_3-20 (Fig. 2). The conformation of ring C was assigned as a distorted chair; the observed coupling constants of H-13 (J=11.4, 1.3 Hz) were in agreement with the equatorial disposition of H-13. The large coupling of 11.4 Hz was a result of a nearly eclipsed arrangement of H-13/H-14, while small couplings were a vicinal coupling for H-13/H-12β and a '*W*' coupling between H-13/H-11α. The small coupling constants of H-12 (dd, J=3.4, 1.3 Hz) and the NOESY cross-peak from H-12 to H-11α, H-11β and H-13 suggested the β-orientation of H-12. The downfield shift of H-9 (δ 1.66) in compound **2** relative to the signal (δ 1.46) in compound **12**[8] was consistent with the deshielding effect of the α-orientated 12-OAc substituent in **2**. An alternative stereochemical possibility for **2**, with a boat orientation of ring C and β-orientation of the acetate group at C-12, was ruled out since in this conformation the axially disposed H-13 would not show a '*W*' coupling with H-11α. In a single-crystal X-ray diffraction of the related compound **13** from the sponge *I. notabilis*, Schmitz et al. showed that all three cyclohexane rings were in chair conformations.[16,21] Accordingly, the new compound **2** was determined to be 12α-acetoxy-17β-hydroxy-15,17-oxidospongian-16-one. This is the first report on the isolation of aplyroseol-type compounds with an acetoxy function at position C-12.

Compound **7** was isolated using reversed-phase HPLC eluting with 100% MeOH and gave a molecular formula of $C_{22}H_{36}O_4$ (HRESMS). Both ^1H and ^{13}C NMR data of **7** were comparable to the known compound **14**, which had been isolated by Dumdei et al. from the nudibranch *Cadlina luteomarginata*.[22] Analysis of the NMR

Table 1
NMR data for compounds **1**–**3** and **5**[a]

C	**1**		**2**		**3**		**5**	
	δ_C[b]	δ_H, m, *J* (Hz)	δ_C[b]	δ_H, m, *J* (Hz)	δ_C[b,7]	δ_H, m, *J* (Hz)[17]	δ_C[b]	δ_H, m, *J* (Hz)[16]
1α	38.5 t	0.92 dt (12.8, 3.4)	38.8 t	0.75 m	38.2 t	0.87 m	38.7 t	0.91
1β		1.68 td (12.8, 2.6)		1.63 m		1.57 m		1.70 td (13.2, 3.0)
2α	18.3 t	1.55 m	18.4 t	1.58 m	18.0 t	1.53 m	18.6 t	1.61 m
2β		1.46 m		1.45 m		1.42 m		1.47 m
3α	41.5 t	1.15 m	41.6 t	1.15 m	41.6 t	1.13 dt (13.1, 4.2)	41.7 t	1.15 dt (13.6, 4.4)
3β		1.40 td (13.7, 2.6)		1.39 m		1.39 m		1.40 td (13.2, 3.0)
4	32.5 s		33.1 s		33.0 s		32.7 s	
5	47.3 d	1.24 dd (13.3, 2.3)	56.5 d	0.98 dd (12.5, 2.8)	56.1 d	0.93 dd (11.8, 2.3)	48.4 d	1.28 dd (13.3, 2.5)
6α	23.6 t	1.88 ddd (15.2, 3.0, 2.6)	19.9 t	1.63 m	18.6 t	1.64 m	24.4 t	1.85 td (14.9, 2.5)
6β		1.55 m		1.35 m		1.37 m		1.60 m
7	70.1 d	5.86 t (3.0)	40.7 t	α 1.15 m, β 1.86 td (13.3, 3.3)	40.8 t	α 1.28 ddt (13.0, 3.8, 1.8),[c] β 1.96 td (13.0, 3.0)	72.9 d	4.73 t (2.9)
8	52.0 s		46.5 s		42.4 s		50.8 s	
9	53.2 d	1.49 m	47.4 d	1.66 dd (14.9, 3.4)	49.6 d	1.33 m	49.4 d	1.52 dd (13.1, 2.8)
10	37.7 s		37.3 s		37.6 s		37.9 s	
11α	16.7 t	1.74 m	22.6 t	1.72 td (13.3, 3.4)	16.9 t	1.64 m	16.0 t	1.45 m
11β		1.74 m		2.30 ddd (14.9, 13.3, 3.4)		1.51 m		1.95 dq (13.1, 4.2)
12α	27.5 t	1.60 m	69.5 d		21.8 t	1.85 m	23.2 t	1.57 m
12β		2.46 qd (13.7, 2.9)		5.54 dd (3.4, 1.3)		1.64 m		2.36 qd (14.1, 2.3)
13	40.6 d	3.25 dt (5.5, 1.9)	43.0 d	2.89 td (11.3, 1.3)	38.8 d	2.89 ddd (10.8, 7.5, 3.7)	37.5 d	2.74 ddd (11.5, 7.6, 1.0)
14	53.4 d	2.67 dd (5.5, 0.7)	47.7 d	2.72 dd (11.3, 6.0)	50.8 d	2.55 dd (7.5, 1.0)[d]	42.1 d	2.85 dd (11.5, 6.0)
15	200.0 d	9.68 d (0.7)	104.4 d	6.09 d (6.0)	177.2 s		104.2 d	6.02 d (6.0)
16	173.5 s		173.2 s		173.2 s		177.0 s	
17	202.4 d	9.97 s	103.8 d	5.49 d (1.8)	72.8 d	α 4.08 d (9.4), β 4.22 dd (9.4, 1.8)[c]	103.5 d	5.44 d (1.9)
18	32.8 q	0.75 s, 3H	33.2 q	0.85 s, 3H	33.2 q	0.81 s, 3H	33.0 q	0.76 s, 3H
19	20.9 q	0.74 s, 3H	21.3 q	0.81 s, 3H	21.3 q	0.86 s, 3H	21.0 q	0.80 s, 3H
20	15.1 q	0.76 s, 3H	15.6 q	0.88 s, 3H	14.3 q	0.73 s, 3H	15.0 q	0.91 s, 3H
$OCOCH_3$	169.3 s		169.5 s				170.2 s	
$OCOCH_3$	20.9 q	2.05 s, 3H	21.2 q	2.06 s, 3H			21.3 q	2.13 s, 3H
$COOCH_3$	51.9 q	3.65 s, 3H			51.7 q	3.70 s, 3H		
OH-17				2.59 d (1.8)				2.77 d (1.9)

[a] Chemical shifts (ppm) referenced to $CDCl_3$ (δ_C 77.0) for carbon and $CHCl_3$ (δ_H 7.24) for proton.
[b] Assignment by HMBC experiments.
[c] H-7α showed a '*W*' coupling (1.8 Hz) to H-17β.
[d] H-14 showed a '*W*' coupling (1.0 Hz) to H-12α due to the boat conformation of ring C.

data confirmed that the new compound **7** lacked an acetoxy group at C-7. A series of 1D NOE experiments revealed that the relative configuration of compound **7** was the same as **14**. Irradiation of H-13 (δ 2.74, td, *J*=12.8, 6.2 Hz) gave NOEs at H-9, H-12α and H-14, which was consistent with the boat conformation of ring C,[8] while irradiation of H-17 (δ 4.96) induced NOEs in H-11β and H_3-20, which suggested a β-orientation of H-17. The α-orientation of 17-OMe was further supported by the downfield shift of H-7β (δ 2.32) due to the deshielding effect of the methoxy group. In comparison, in compound **2** in which the 17-OH is β-orientated, the resonance for H-7 is at δ 1.86.

Compound **7** was considered to be an artefact of an aldehyde **15**, the probable natural product, which likely exists in equilibrium with the hemiacetal **16**. In a synthetic study exploring the reduction of **10**, Arnó et al. had shown that the cyclic hemiacetal **16** was more stable and the ring-opened **15** was not detected in the ^{1}H NMR spectrum.[19] During the purification of the nudibranch metabolites in our study, the parent aldehyde **15** reacted with MeOH present in the chromatography solvent system to give compound **7**.

To confirm the origin of compound **7**, a small portion of the crude extract was exposed briefly to MeOH-d_4, which resulted in the isolation of the deuterated compound **17**. The carbon resonance of the 17-OCD_3 in **17** was upfield by 0.9 ppm due to the isotopic shift caused by the CD_3 group.[23] This experiment showed that compound **7**, and possibly **14**, can be isolated during extraction and are likely artefacts. We noted that compound **14** had been isolated by Dumdei et al. using an extraction procedure that included MeOH/CH_2Cl_2.[22]

Synthetic studies have also shown that the dialdehydes **10** and **11** have a high tendency for lactone-hemiacetal formation (e.g., formation of compound **12** from **10**), due to the higher stability of the cyclic hemiacetals.[19,20] Prior to our study, the dialdehyde metabolites **10** and **11** had never been isolated before from natural sources, although it has been suggested that these compounds were key intermediates for the formation of other oxygenated diterpenes such as the aplyroseols.[24] When we exposed dialdehyde **1** briefly to MeOH-d_4 in an NMR tube to monitor its stability, the ^{1}H NMR spectrum showed evidence of cyclisation to acetal products.

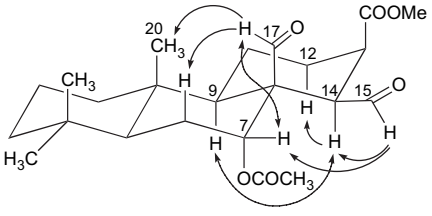

Figure 1. Selected NOE correlations observed for compound **1**.

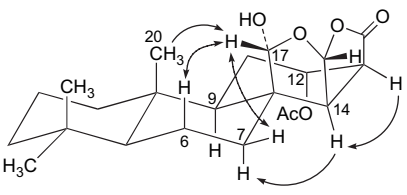

Figure 2. Selected NOE correlations observed for compound **2**.

Subsequent purification led to the isolation of the two deuterated compounds **8** and **9** (confirmed by HRESMS).

The NMR spectra of compound **8** were similar to those of **1**, except for the absence of both aldehyde signals and the presence of two new acetal signals (δ_C 101.1; δ_H 5.70 and δ_C 107.4; δ_H 4.83). A large coupling constant between H-12β and H-13 (13.7 Hz) indicated a diaxial arrangement between the two protons, and hence a boat conformation in ring C.[8] Both 15-OCD_3 and 17-OH groups were α-orientated, as determined from 2D NOESY cross-peaks of H-15/H-12β, H-17/H-11β and OH-17/H-7. Furthermore, the resonance of H-7 (δ 5.25) of **8** was shifted downfield compared to that of compound **5** (δ_H 4.73),[16] which had the β-orientated 17-OH. Compound **9** exhibited the same molecular formula as compound **8**; comparison of the NMR data and careful analysis of the HMBC spectra confirmed that **9** was the regioisomer of **8**, with the positions of the OCD_3 and OH groups in the hemiacetal ring interchanged. 2D NOESY data showed that compound **9** had the same relative configuration as **8**.

The absolute configuration of (+)-isoagatholactone (**18**), a related compound of (+)-spongian-16-one (**6**), has been defined by total synthesis from (+)-manool.[25] By comparison of their $[\alpha]_D$ values, the four closely related compounds isolated from this study (**2**, **3**, **6**, and **7**) were assigned to the same enantiomeric series as compound **18**. The absolute configuration of (−)-aplyroseol-1 (**13**) has been established from a single-crystal X-ray analysis of its *p*-bromobenzoyl derivative.[26] Therefore, it is reasonable to assume that the isolated compounds **1**, **4**, and **5**, all exhibiting negative $[\alpha]_D$ values, have the same absolute stereochemistry as compound **13**. Introduction of the 7-OAc group appears to change the sign of the $[\alpha]_D$, as in the case of compounds **4**, **5**, and **14**, which all have negative $[\alpha]_D$, compared to their counterparts without the 7-OAc group (**3**, **2**, and **7**, respectively) that all show a positive $[\alpha]_D$.

^{1}H NMR spectra of extracts obtained from the mantle and internal organs showed the presence of diterpene metabolites. Characteristic signals in the ^{1}H NMR spectrum showed the presence of known metabolites **3**–**5** in the internal organs, while metabolite **1** was only present in a trace amount. Although there was no evidence from ^{1}H NMR data for the presence of compound **2** or **6** in the internal organs, these compounds might still be present. The mantle extract on the other hand contained a high percentage of the dialdehyde **1** since its characteristic signals were dominant in the ^{1}H NMR. The accumulation of dialdehyde **1** in the mantle suggests that this metabolite may have an ecological role in the nudibranch, possibly as a feeding deterrent.[11] Some naturally occurring dialdehydes having diterpenoid and sesquiterpenoid skeletons had been shown to possess potent antifeedant activities against insects and fish.[27,28] Based on the pattern of metabolites from this particular nudibranch, it is tempting to suggest that the dietary origin of these oxidised diterpenes could be dendroceratid sponges.[3]

All of the isolated compounds were screened for P388 cytotoxicity. Compounds **1**, **2**, **5** and **8** showed moderate activity, with IC_{50} values of 4.13, 2.72, 2.72 and 1.21 μg/mL, respectively.

3. Conclusions

This study reported two new diterpenes **1** and **2** along with four known metabolites **3**–**6** previously isolated from dendroceratid sponges and from the nudibranch *C. obsoleta*. The mantle showed a higher concentration of the dialdehyde **1**, suggesting a possible ecological role of this metabolite, although further studies are needed to confirm this. The dialdehyde metabolite **1** had a high tendency for lactone-hemiacetal formation, and exposure to MeOH during isolation may lead to facile conversion to cyclic hemiacetals as shown by an NMR study.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained using a Perkin–Elmer 241-MC polarimeter. 1D and 2D NMR spectra were acquired using Bruker DRX-500 or Bruker DMX-750 instrument. NMR spectra were obtained in deuterochloroform at room temperature, and were internally referenced to $CHCl_3$ at δ_H 7.24 or $CDCl_3$ at δ_C 77.0. Positive ion electrospray mass spectra (LRESMS) were determined using a Bruker Esquire HCT instrument or (HRESMS) using a MicroTof Q instrument each with a standard ESI source. Samples were introduced into the source using MeOH as solvent. Normal phase HPLC was carried out using a Waters 515 pump with a Waters 10μ μPorasil 7.8×300 mm column and a Gilson 132 series RI detector with EtOAc/hexanes as solvent. Reverse phase HPLC was carried out using a Shimadzu LC-20AT pump with a Phenomenex Gemini 5μ C18 10×250 mm column, and a Shimadzu ELSD-LT (low temperature evaporative light scattering detector).

4.2. Biological material

A mollusc specimen provisionally identified as *Chromodoris* sp. was collected from Coral Gardens dive site at the Inner Gneerings Reef, a group of shoals near Mooloolaba (Australia), using SCUBA at a depth of 10–15 m on 25 March 2007. The sample was taken back to the laboratory and stored at −20 °C until extraction.

4.3. Extraction and isolation of diterpenes

The specimen of *Chromodoris* sp. (frozen weight 13.4 g) was dissected into mantle (1.7 g) and internal organs (0.88 g). Each section was extracted exhaustively with acetone using sonication. The extract was filtered through cotton wool and concentrated under reduced pressure to give an aqueous residue, which was partitioned with EtOAc. The EtOAc fraction was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give 45.6 mg crude extract (orange oil) from the mantle and 12.7 mg crude extract (yellow oil) from the internal organ. The mantle extract was subjected to SiO_2 flash chromatography with gradient elution (hexanes→EtOAc) to give eight fractions (A–G). Fraction D (1.47 mg) eluting in hexanes/EtOAc (5:1) was subjected to NP-HPLC (hexanes/EtOAc, 80:20) to give the known compounds **6** (0.32 mg) and **3** (0.50 mg). Fraction E (14.4 mg) eluting in hexanes/EtOAc (3:1) was purified using RP-HPLC (100% MeOH) to yield compound **7** (1.1 mg). Fraction F (16.2 mg, hexanes/EtOAc, 2:1) was also subjected to the same NP-HPLC conditions to afford compounds **4** (4.1 mg), **1** (4.5 mg), **2** (1.0 mg) and **5** (2.32 mg) in order of elution.

*4.3.1. Methyl 7α-acetoxy-8β,14β-diformylpodocarpane-13β-carboxylate (**1**)*

Colourless oil; $[\alpha]_D$ −13.4 (*c* 0.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; HRESMS *m*/*z* 429.2254 $[M+Na]^+$ (calcd for $C_{23}H_{34}O_6Na$, 429.2253).

*4.3.2. 12α-Acetoxy-17β-hydroxy-15,17-oxidospongian-16-one (**2**)*

Colourless oil; $[\alpha]_D$ +14.0 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; HRESMS *m*/*z* 415.2093 $[M+Na]^+$ (calcd for $C_{22}H_{32}O_6Na$, 415.2097).

*4.3.3. Dendrillol-3 (**3**)*[17]

Colourless oil; $[\alpha]_D$ +41.9 (*c* 0.10, $CHCl_3$), this value was not reported previously in the literature; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; LRMS *m*/*z* 371.2 $[M+Na]^+$.

*4.3.4. Methyl 15,17-epoxy-17α-methoxy-ent-isocopalan-16-oate (**7**)*

Colourless oil; $[\alpha]_D$ +29.2 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.96 (1H, s, H-17), 3.82 (1H, dd, *J*=9.6, 6.2 Hz, H-15α), 3.63 (3H, s, $COOCH_3$), 3.55 (1H, dd, *J*=9.6, 1.5 Hz, H-15β), 3.34 (3H, s, 17-OCH_3), 2.74 (1H, td, *J*=12.8, 6.2 Hz, H-13), 2.32 (1H, td, *J*=13.0, 3.3 Hz, H-7α), 2.20 (1H, dt, *J*=6.2, 1.5 Hz, H-14), 1.81–1.75 (2H, m, H_2-12), 1.62–1.58 (1H, m, H-1β), 1.61–1.57 (1H, m, H-11α), 1.59–1.56 (1H, m, H-2α), 1.48 (1H, qd, *J*=13.6, 2.3 Hz, H-6α), 1.42–1.34 (2H, m, H-2β and H-11β), 1.40–1.36 (1H, m, H-6β), 1.38–1.34 (2H, m, H-3β and H-9), 1.20 (1H, dt, *J*=12.8, 4.2 Hz, H-7β), 1.13 (1H, dt, *J*=13.0, 4.1 Hz, H-3α), 0.90 (1H, dd, *J*=12.5, 2.3 Hz, H-5), 0.87–0.82 (1H, m, H-1α), 0.84 (3H, s, H_3-20), 0.83 (3H, s, H_3-18), 0.79 (3H, s, H_3-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.5 (s, C-16), 105.6 (d, C-17), 65.7 (d, C-15), 56.4 (d, C-5), 55.2 (q, 17-O*C*H_3), 51.4 (q, COO*C*H_3), 50.4 (d, C-14), 49.5 (d, C-9), 49.0 (s, C-8), 41.8 (t, C-3), 39.1 (d, C-13), 38.8 (t, C-1), 37.9 (s, C-10), 36.8 (t, H-7), 33.3 (q, C-18), 32.9 (s, C-4), 21.4 (d, C-19), 20.6 (t, C-6), 18.9 (t, C-12), 18.2 (t, C-2), 15.6 (t, C-11), 13.8 (q, C-20); HRESMS *m*/*z* 387.2518 $[M+Na]^+$ (calcd for $C_{22}H_{36}O_4Na$, 387.2511).

4.4. NMR study of methyl 7α-acetoxy-8β,14β-dioxopodocarpan-13β-oate (1)

Compound **1** (3.0 mg) was dissolved in MeOH-d_4 (0.4 mL) and the formation of cyclic products monitored by ^1H NMR. The sample was later dried under nitrogen, followed by purification with NP-HPLC (hexanes/EtOAc, 75:25) to afford the deuterated compounds **8** (1.5 mg) and **9** (0.4 mg).

*4.4.1. Compound **8***

Colourless oil; $[\alpha]_D$ −29.3 (*c* 0.15, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.70 (1H, d, *J*=9.8 Hz, H-17), 5.25 (1H, dd, *J*=3.5, 2.2 Hz, H-7), 4.83 (1H, d, *J*=0.7 Hz, H-15), 3.66 (3H, s, $COOCH_3$), 2.90 (1H, d, *J*=9.8 Hz, OH-17), 2.75 (1H, td, *J*=13.7, 5.0 Hz, H-13), 2.57 (1H, dd, *J*=5.0, 0.7 Hz, H-14), 2.08 (3H, s, $OCOCH_3$), 1.86 (1H, ddd, *J*=14.8, 3.5, 2.5 Hz, H-6α), 1.80–1.76 (1H, m, H-12α), 1.73 (1H, ddd, *J*=14.8, 13.5, 2.2 Hz, H-6β), 1.71–1.59 (2H, m, H-1β and H-12β), 1.66–1.61 (1H, m, H-9), 1.64–1.59 (1H, m, H-11α), 1.63–1.59 (1H, m, H-2α), 1.46–1.40 (1H, m, H-2β), 1.42–1.38 (1H, m, 3β), 1.35–1.29 (1H, m, H-11β), 1.28 (1H, dd, *J*=13.5, 2.5 Hz, H-5), 1.15 (1H, dt, *J*=13.5, 3.8 Hz, H-3α), 0.92 (3H, s, H_3-20), 0.89 (1H, dt, *J*=13.3, 4.1 Hz, H-1α), 0.77 (3H, s, CH_3-19), 0.75 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.0 (s, C-16), 169.9 (s, O*C*OMe), 107.4 (d, C-15), 101.1 (d, C-17), 74.9 (d, C-7), 54.4 (q, 15-OCD_3), 51.7 (q, COO*C*H_3), 51.6 (s, C-8), 51.3 (d, C-14), 48.3 (d, C-5), 44.4 (d, C-9), 41.6 (t, C-3), 39.0 (t, C-1), 37.9 (s, H-10), 37.5 (d, H-13), 33.2 (q, C-18), 32.4 (s, C-4), 24.4 (t, C-6), 21.4 (q, $OCOCH_3$), 21.1 (q, C-19), 19.2 (t, C-12), 18.4 (t, C-2), 14.7 (q, C-20), 14.3 (t, C-11); HRESMS *m*/*z* 464.2699 $[M+Na]^+$ (calcd for $C_{22}H_{35}D_3O_7Na$, 464.2704).

*4.4.2. Compound **9***

Colourless oil; $[\alpha]_D$ −27.9 (*c* 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.40 (1H, dd, *J*=3.7, 2.0 Hz, H-7), 5.25 (1H, s, H-17), 5.16 (1H, br d, *J*=8.4 Hz, H-15), 3.67 (3H, s, $COOCH_3$), 2.84 (1H, d, *J*=8.4 Hz, OH-15), 2.74 (1H, td, *J*=13.5, 5.0 Hz, H-13), 2.56 (1H, br d, *J*=5.0 Hz, H-14), 2.08 (3H, s, $OCOCH_3$), 1.82 (1H, ddd, *J*=14.5, 3.7, 2.4 Hz, H-6α), 1.81–1.77 (1H, m, H-12α), 1.71–1.66 (1H, m, H-12β), 1.66–1.60 (1H, m, H-6β), 1.67–1.59 (2H, m, H-1β and H-9), 1.63–1.58 (1H, m, H-11α), 1.61–1.55 (1H, m, H-2α), 1.45–1.37 (1H, m, H-2β), 1.42–1.38 (1H, m, 3β), 1.34–1.28 (1H, m, H-11β), 1.29 (1H, dd, *J*=13.6, 2.4 Hz, H-5), 1.15 (1H, dt, *J*=12.8, 3.5 Hz, H-3α), 0.90–0.82 (1H, m, H-1α), 0.87 (3H, s, H_3-20), 0.78 (3H, s, CH_3-19), 0.74 (3H, s, H_3-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.9 (s, C-16), 170.1 (s, O*C*OMe), 107.0 (d, C-17), 100.8 (d, C-15), 75.5 (d, C-7), 55.0 (q, 17-OCD_3), 53.6 (d, C-14), 51.8 (q, COO*C*H_3), 51.6 (s, C-8), 48.3 (d, C-5), 44.4 (d, C-9), 41.5 (t, C-3), 38.8 (t, C-1), 37.9 (s, H-10), 37.8 (d, C-13), 33.0 (q, C-18), 32.4 (s, C-4), 24.6 (t, C-6), 21.3 (q, $OCOCH_3$), 21.1 (q, C-19), 19.0 (t, C-12), 18.2 (t, C-2), 14.5 (t, C-11), 14.3 (q, C-20); HRESMS *m*/*z* 464.2688 $[M+Na]^+$ (calcd for $C_{22}H_{35}D_3O_7Na$, 464.2704).

4.5. Preparation and isolation of compound 17

During the dissection of the mollusc, some mucus was separated from the animal. The mucus was extracted with acetone and processed according to the procedure described above for the mantle and internal organs to give a crude EtOAc extract (orange oil, 19.3 mg). The ^1H NMR spectrum ($CDCl_3$) of the crude EtOAc extract was identical with the spectrum obtained from the mantle extract. The mucus crude EtOAc extract was dried, then redissolved in MeOH-d_4 (0.5 mL) and dried again under nitrogen, followed by NP-HPLC purification with hexanes/EtOAc (80:20) to give compound **17**.

*4.5.1. Compound **17***

Colourless oil; $[\alpha]_D$ +33.8 (*c* 0.04, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz), identical to compound **7**, except that signal at δ_H 3.34 (3H, s, 17-OCH_3) was absent; ^{13}C NMR ($CDCl_3$, 125 MHz), identical to compound **7**, except for 17-OCD_3 (δ_C 54.3); LRESMS *m*/*z* 390.3 $[M+Na]^+$; HRESMS *m*/*z* 333.2411 $[M-OCD_3]^+$ (calcd for $C_{21}H_{33}O_3$, 333.2424).

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