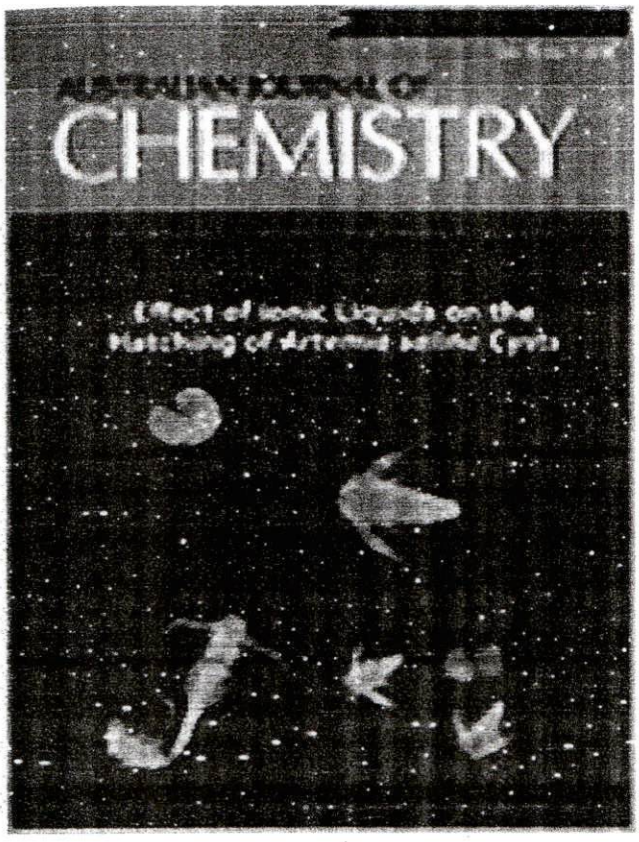
CHEMISTRY

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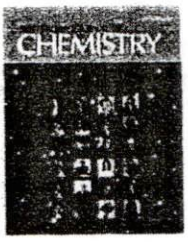

Australian Journal of Chemistry
Volume 64 Number 6 2011
RESEARCH FRONT: Women in Chemistry

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Women in Chemistry

Jennifer M. Bennett

International Year of
CHEMISTRY
2011

2011 is the International Year of Chemistry. It marks a worldwide celebration of the achievements of chemistry and its contributions to the well being of humankind. The year 2011 also coincides with the 100th anniversary of the Nobel Prize in Chemistry being awarded to Madame Marie Curie. This special issue is a fitting opportunity to celebrate the contributions of women to science.

Full Text | PDF (217 KB)

Women in Chemistry in Australia: From a Slow Start to a More Promising Future

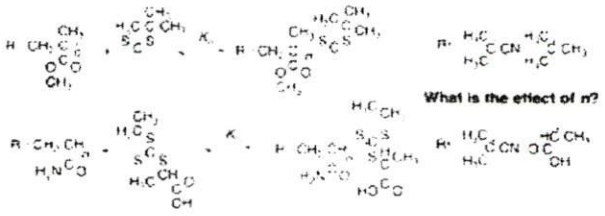
Margaret M. Sheil

Abstract | PDF (356 KB) - $25.00 BUY NOW

An *Ab Initio* Investigation of the Chain-Length Dependence of the Addition–Fragmentation Equilibria in RAFT Polymerization

Ching Yeh Lin and Michelle L. Coote

pp. 747-756



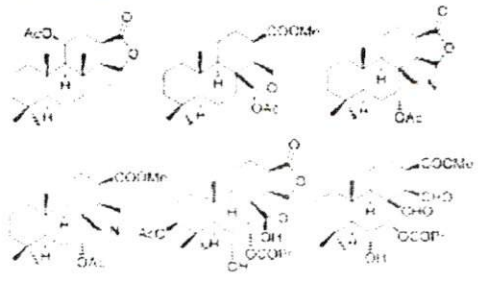
Significant and variable chain length effects on the addition–fragmentation equilibrium constant in RAFT polymerization extend to at least the trimer stage. They arise through primarily the stability of the attacking radical, but with significant contributions from homoanomeric effects and hydrogen-bonding interactions.

Abstract | PDF (293 KB) - $25.00 BUY NOW | Supplementary Material (1.3 MB)

Structures and Anatomical Distribution of Oxygenated Diterpenes in the Australian Nudibranch *Chromodoris reticulata*

Suciati, Lynette K. Lambert and Mary J. Garson

pp. 757-765



Six new diterpene metabolites have been isolated from the nudibranch *Chromodoris reticulata* (Chromodorididae), along with 17 known diterpenes, and their anatomical distribution investigated. Aplyroseol-2 was the major compound in the mantle tissue along with some dialdehydes, while ambliofuran was the only diterpene found solely in the internal organs. The presence of lactone-acetal-hemiacetal functionality in many of the isolated compounds is a consequence of reactive dialdehyde intermediates present in the mollusc.

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Analysis of Thiol-sensitive Core-cross-linked Polymeric Micelles Carrying Nucleoside Pendant Groups using 'On-line' Methods: Effect of Hydrophobicity on Cross-linking and Degradation

Bianca M. Blunden, Donald S. Thomas and Martina H. Stenzel

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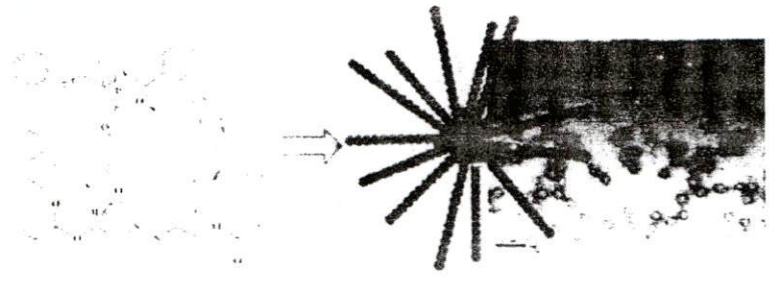

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Aust. J. Chem. **2011**, *64*, 757–765

Structures and Anatomical Distribution of Oxygenated Diterpenes in the Australian Nudibranch *Chromodoris reticulata*

Suciati,[A,B] Lynette K. Lambert,[C] and Mary J. Garson[A,D]

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The structures and stereochemistry of six new diterpenes (**1–6**), two of which contain cyclic imine functionality, have been deduced by 2D NMR spectroscopy. The anatomical distribution of these, and of 17 other diterpenes (**7–23**) that were also isolated, has been investigated. The known compound aplyroseol-2 (**14**) was the major compound in the mantle tissue along with some dialdehydes, while the linear furan ambliofuran (**7**) was the only diterpene found solely in the internal organs. The presence of lactone-acetal-hemiacetal functionality in many of the isolated compounds is a consequence of the reactive dialdehydes present in the mollusc.

Manuscript received: 21 January 2011.
Manuscript accepted: 16 March 2011.

Introduction

Nudibranchs from the order of Opisthobranchia (Mollusca: Gastropoda) are shell-less marine molluscs which lack physical defences against predation; and thus, may employ chemical defences to deter predators. They may be protected by metabolites obtained from dietary sources, commonly sponges, or from *de novo* biosynthesis.[1–4] For example, a predator–prey interaction has been determined for *Glossodoris atromarginata* and its sponge diet that involves sesterterpene metabolites.[5] Additionally, isotope-feeding studies have revealed the capability of dorid nudibranchs such as *Dendrodoris limbata* to produce terpenoids from mevalonic acid via *de novo* biosynthesis.[6–8]

Numerous diterpenes have been reported from chromodorid nudibranchs,[1,2,9] and some of these metabolites show pronounced biological activity.[7,8,10–13] Molinski and Faulkner isolated the aromatic norditerpenes macfarlandins A and B,[14] closely related to aplysulfurin from *Aplysilla sulfurea*,[15,16] and macfarlandins C–E, related to metabolites found in *Dendrilla* sp.,[17] from *Chromodoris macfarlandi*. Their findings strongly suggested that *C. macfarlandi* may prey on two different sponges.[18] The Golgi-modifying properties of macfarlandin

Professor Mary Garson is a Professor of Chemistry at The University of Queensland. She graduated from The University of Cambridge, UK with a Ph.D. (1977) and MA (1978), after which she undertook postdoctoral studies funded by a Royal Society of London Overseas Research Fellowship at the Universita Cattolica, Rome (1978). She then returned to Cambridge as a college research fellow and tutor at New Hall (since renamed as Murray Edwards College) within the university. Next she worked as a medicinal chemist in the UK pharmaceutical industry before emigrating to Australia following the award of a Queen Elizabeth II Research Fellowship at James Cook University of North Queensland (1983–1986). Prior to joining The University of Queensland as a lecturer in 1990, she held a lecturing position at The University of Wollongong in NSW. In her academic research, Professor Garson has made distinguished contributions to the fields of terrestrial and marine natural products, biosynthesis, and chemical ecology over a 30-year period. She is widely recognized for her collaborative research with colleagues from South-East Asian countries, including Thailand, Indonesia, and the Philippines. Professor Garson has been Chair of the International Relations Committee, as well as President of the Queensland branch, of the Royal Australian Chemical Institute. She was Executive Secretary of the team organizing the World Chemistry Congress in Brisbane in 2001, and is currently co-chair of the organizing committee for the 27th International Symposium on the Chemistry of Natural Products. From 2002 to 2004, she was chair of Australian Science Innovations (previously known as the Australian Science Olympiads). She is currently a Titular member, and honorary Secretary, of Division III (organic and biomolecular) of IUPAC, and the Division proposes to appoint her as Vice President (2012–13) succeeding to the Division Presidency in 2014–2015. An unusual form of professional recognition is that a new species of marine flatworm that she first collected at Heron Island has been named Maritigrella marygarsonae.

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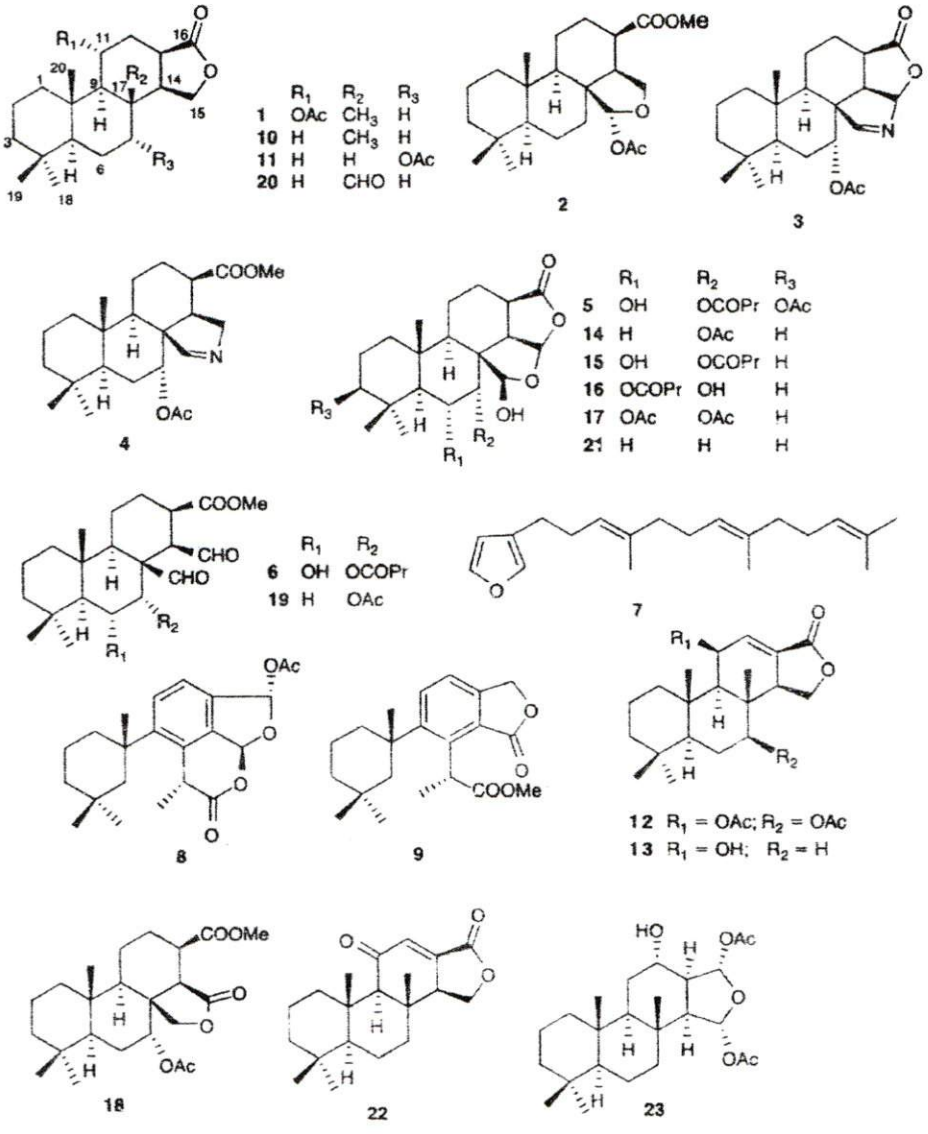

Fig. 1. Structures of diterpenoid metabolites isolated in the current study on the nudibranch *Chromodoris reticulata*.

E and of a synthetic analogue in which the hydroazuleno moiety is replaced by a *tert*-butyl group have been reported.[19] Cell biological studies of the dynamics of Golgi organization are providing fundamental insight into how these organelles play a key role in protein modification within the cell. An intriguing question to address is how this biological activity may relate to the ecological roles of macfarlandin E in both the mollusc and its dietary sponge.

We report six new diterpenes (**1–6**) together with 17 known diterpenes (**7–23**) (Fig. 1) isolated from two specimens of *Chromodoris reticulata*. The anatomical distribution of the diterpene compounds within the various tissue types of the mollusc, was explored by dissection and analysis of one of the two specimens. The data are compared with our study[20] on a specimen of a *Chromodoris* mollusc (species taxonomy possibly *reticulata*) which had earlier provided the two diterpenes **19** and **24**, along with the four known metabolites **10**, **14**, **18**, and **25** (Figs 1 and 2).

Results and Discussion

Two large specimens of *Chromodoris reticulata* were collected by SCUBA from the Gneerings Reef, offshore from Mooloolaba, in South East Queensland. One nudibranch was extracted with acetone to investigate the total chemistry and gave a terpene-rich organic extract that was fractionated by silica flash chromatography (hexanes/EtOAc), followed by normal phase HPLC (NP-HPLC) using hexanes/EtOAc. Using these separation protocols, three new (**1–3**) and 15 known metabolites were obtained. The known metabolites were identified by comparison of NMR data with the literature; these were ambliofuran (**7**),[21–23] aplysulfurin (**8**),[15,16] membranolide (**9**),[24]

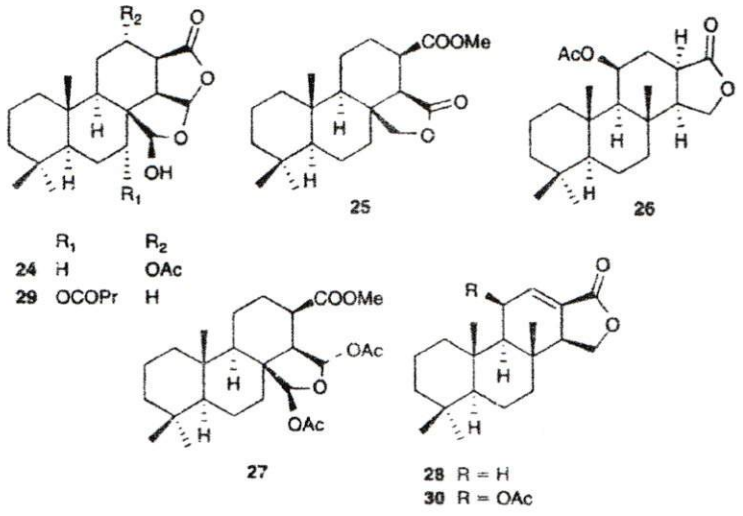

Fig. 2. Structures of diterpenoid metabolites isolated in earlier sponge or nudibranch studies, or from synthetic transformations.

spongian-16-one (**10**),[25] 7α-acetoxy-spongian-16-one (**11**),[22] dorisenone D (**12**),[13] 11β-hydroxyspongi-12-en-16-one (**13**),[26] aplyroseols-2, 3 and 5 (**14–16**),[13,22,27] dendrillol-2 (**17**),[23] aplyroseol-9 (=7α-acetoxydendrillol-3) (**18**),[28] the aldehydes **19**[20] and **20**,[29] and dendrillol-1 (**21**).[23] Diterpenes **10**, **14**, and **18** were isolated in our earlier study on an unidentified chromodorid nudibranch.[20] The second nudibranch was dissected to separate the mantle tissue from the internal organs in order to probe the distribution of individual metabolites. Extraction of the mantle tissue using the same procedure as for the first sample resulted in the isolation of three new diterpenes (**4–6**), while two additional compounds, namely spongi-12-en-11,16-dione (**22**)[26] and 12-deacetyl-aplysillin (**23**),[30] were identified by comparison with literature data. All compounds isolated from the first specimen were also isolated from the second specimen.

Diterpene **1** displayed a sodiated molecular ion peak in the HR-ESI MS at *m/z* 385.2345, corresponding to the molecular formula $C_{22}H_{34}O_4$. Inspection of the NMR spectra (Tables 1 and 2) confirmed the presence of four methyl singlets (δ_H 0.93, 0.91, 0.86, 0.82), and an acetate methyl (δ_H 2.01), while two oxymethylene protons (δ_H 4.31) were linked by HSQC to a carbon at δ_C 67.6, and showed HMBC correlations to a carbonyl at δ_C 180.8. These data suggested a spongian-16-one substituted with an acetate group, but when compared with 7α-acetoxy-spongian-16-one (**11**),[22] the shift of the oxymethine proton (δ_H 5.17 for H11 in **1** compared with δ_H 4.92 for H7 of **11**[22]) supported a different substitution pattern. The 11-OAc was deduced by HMBC correlations from H9 and H12α to C11, and confirmed by 1D TOCSY and COSY experiments. A boat conformation was inferred for ring C, since H13 at δ_H 2.90 showed a large coupling (*J* 11.5 Hz) to H12β and so was axially-orientated. Additionally, H14 and H13 were eclipsed, also showing an 11.5 Hz coupling; while an NOE between H12β and Me-17 supported the proposed conformation.[20,29,31] In an alternative 'flattened' chair conformation, there would not be a large coupling between the equatorial H13 and either H12 proton, nor would an NOE between H12β and Me-17 be expected. Although such data must be used with care, an NOE between H9 and H14, but no equivalent NOE between H11 and H13, suggested that the 11-OAc was α-oriented. NOEs between H11/Me-20 and between H11/H1β agreed with this interpretation. The β orientation of H11 was consistent with a *J* of 5.0 Hz between H11 and H9; a dihedral angle of 127.3° was suggested by molecular modelling (*Chem Bio 3D Ultra* 12.0 (Cambridge)) using a MM2 force field for energy minimization to an RMS of 0.100). The data for **1** differed with the partial data reported for the synthetic **26** (with a β-OAc), prepared by hydrogenation of a diterpene isolated from *Spongia officinalis*.[26] Although we named **1** as 11α-acetoxyspongian-16-one in view of its relationship to the known **10**, we note that the 'spongianone' nomenclature prevalent in the literature for such lactones is unsatisfactory.

Diterpene **2** was obtained by NP-HPLC (hexanes/EtOAc, 80/20) and had the molecular formula of $C_{23}H_{36}O_5$ inferred from HR-ESI MS (*m/z* 415.2463 $[M+Na]^+$). The ^{1}H NMR spectrum showed the presence of three methyl singlets (δ_H 0.85, 0.83, 0.78), an acetate methyl (δ_H 2.11), a carboxymethyl (δ_H 3.65), an acetal proton (δ_H 6.44), and two oxymethylene protons (δ_H 3.95, 3.73). The data were similar to those of diterpene **27**, except for the oxymethylene proton signals instead of signals for a second acetal moiety.[29] HMBC correlations from the acetal proton (δ_H 6.44) to C7 (δ_C 37.4) and the OAc (δ_C 170.8) confirmed that the acetate group was attached to C17. The relative configuration of **2** was then explored.[20,31] A boat conformation was inferred for ring C since the axial H13 at δ_H 2.79 again showed a large coupling (*J* 12.3 Hz) to H12β. A 5.7 Hz coupling between H13 and H14 matched with a dihedral angle of approximately 60°,[29] while the NOE between H9 and H13 agreed with the measured inter proton distance of 2.2 Å. Inspection of molecular models revealed that H17 would show an NOE to Me-20 irrespective of the C17 configuration. However, there was a strong NOE from H17 to the H15 proton (δ_H 3.73) assigned as β owing to the small coupling (1.7 Hz) with H14 that results from a dihedral angle close to 90°. The β orientation of H17 was further confirmed by an NOE to H11β, and by the shifts of H7α and H7β (δ_H 1.36 and 2.35) that matched equivalent values in diterpene **27** (δ_H 1.39 and 2.54). This latter metabolite was previously isolated from the nudibranch *Ceratosoma brevicaudatum*,[29] now considered a species of the genus *Chromodoris*.[2]

Table 1. ^{1}H NMR data for diterpenes 1–6[A]

C	1 δ_H, m (J in Hz)[B]	2 δ_H, m (J in Hz)[B]	3 δ_H, m (J in Hz)[B]	4 δ_H, m (J in Hz)[C]	5 δ_H, m (J in Hz)[B]	6 δ_H, m (J in Hz)[D]
1	α 0.90 m	α 0.86 m	α 0.95 td (13.9, 3.8)	α 0.95 m	α 1.15 m	α 0.97 m
	β 1.43 m	β 1.64 m	β 1.77 m	β 1.67 m	β 1.76 m	β 1.67 dt (13.2, 3.3)
2	α 1.59 m	α 1.60 m	α 1.65 m	α 1.63 m	α 1.73 m	α 1.56 m
	β 1.38 m	β 1.42 m	β 1.52 m	β 1.48 m	β 1.65 m	β 1.47 m
3	α 1.13 td (13.5, 4.0)	α 1.14 td (13.3, 3.6)	α 1.21 td (13.3, 4.0)	α 1.21 td (13.5, 3.5)	4.50 dd (11.8, 4.8)	α 1.22 td (13.7, 3.9)
	β 1.38 m	β 1.38 m	β 1.47 m	β 1.44 m		β 1.38 dt (13.7, 3.3)
5	0.89 brd (11.5)	0.93 dd (12.3, 2.3)	1.42 dd (11.9, 2.0)	1.39 dd (13.5, 2.5)	1.60 d (11.3)	1.47 d (11.7)
6	α 1.60 m	α 1.56 m	α 1.96 ddd (14.7, 3.1, 2.0)	α 1.99 dt (15.0, 3.5)	4.26 ddd (11.3, 6.0, 2.6)	4.09 ddd (11.7, 4.3, 3.0)
	β 1.41 m	β 1.15 m	β 1.56 m	β 1.68 m		
7	α 1.03 td (12.0, 4.0)	α 1.36 m	4.60 t (3.1)	4.75 t (2.5)	4.95 d (2.6)	6.07 d (3.0)
	β 1.70 dt (12.0, 3.5)	β 2.35 m				
9	1.17 d (5.0)	1.40 m	1.46 dd (11.9, 2.3)	1.64 m	1.46 dd (13.5, 2.9)	1.46 dd (12.3, 2.5)
11	5.17 td (5.0, 2.0)	α 1.63 m	α 0.77 m	α 0.98 m	α 1.45 m	1.72 m; 1.76 m
		β 1.43 m	β 1.66 m	β 1.60 m	β 1.98 qd (13.5, 4.4)	
12	α 2.17 ddd (16.0, 8.5, 2.0)	1.83 m	α 1.74 m	1.80 m	α 1.58 m	α 1.61 m
	β 1.98 ddd (16.0, 11.5, 5.0)		β 2.21 m		β 2.39 ddd (13.9, 4.4, 2.4)	β 2.47 dq (13.6, 2.0)
13	2.90 td (11.5, 8.5)	2.79 dt (12.3, 5.7)	2.72 ddd (12.0, 9.1, 2.6)	2.74 m	2.76 m	3.29 td (5.6, 2.0)
14	2.31 m	2.34 m	2.92 dd (12.0, 6.6)	2.47 m	2.76 m	2.56 d (5.6)
15	4.31 m	α 3.95 dd (9.7, 6.9)	6.30 dd (6.6, 1.3)	α 4.00 ddd (17.0, 9.0, 2.0)	6.06 m	9.73 s
		β 3.73 dd (9.7, 1.7)		β 3.71 ddd (17.0, 6.0, 2.5)		
17	0.91 s, 3H	6.44 s	7.99 d (1.3)	7.57 br s	5.53 d (1.8)	10.00 s
18	0.86 s, 3H	0.85 s, 3H	0.81 s, 3H	0.80 s, 3H	1.10 s, 3H	1.08 s, 3H
19	0.82 s, 3H	0.78 s, 3H	0.83 s, 3H	0.83 s, 3H	1.05 s, 3H	0.95 s, 3H
20	0.93 s, 3H	0.83 s, 3H	1.03 s, 3H	0.89 s, 3H	1.01 s, 3H	0.85 s, 3H
$OCOCH_3$	2.01 s, 3H	2.11 s, 3H	2.16 s, 3H	2.11 s, 3H	2.07 s, 3H	
$COOCH_3$		3.65 s, 3H		3.67 s, 3H		3.67 s, 3H
$OCOCH_2CH_2CH_3$					2.45 m	2.38 m
$OCOCH_2CH_2CH_3$					1.73 m	1.68 m
$OCOCH_2CH_2CH_3$					1.01 t (7.4), 3H	0.98 t (7.4), 3H
6-OH					1.44 d (6.0)	1.56[E]
17-OH					2.87 d (1.8)	

[A]Chemical shifts [ppm] referenced to $CHCl_3$ (δ_H 7.26).
[B]Data recorded at 500 MHz.
[C]Data recorded at 750 MHz.
[D]Data recorded at 900 MHz.
[E]Detected in DQFCOSY by correlation to H6.

Table 2. ^{13}C NMR data for diterpenes 1–6[A,B]

C	1 δ_C[C]	2 δ_C[C]	3 δ_C[C]	4 δ_C[D]	5 δ_C[C]	6 δ_C[E]
1	38.5 t	39.6 t	38.9 t	38.9 t	37.1 t	39.1 t
2	18.2 t	18.6 t	18.5 t	18.4 t	23.5 t	18.7 t
3	42.1 t	42.1 t	41.9 t	42.0 t	80.3 d	43.4 t
4	33.5 s	33.6 s	33.0 s	32.8 s	37.8 s	33.4 s
5	56.9 d	56.9 d	48.0 d	48.7 d	52.5 d	52.0 d
6	18.2 t	20.7 t	25.3 t	25.6 t	69.9 d	69.3 d
7	42.2 t	37.4 t	73.8 d	75.2 d	77.0 d	73.9 d
8	35.7 s	49.6 s	63.2 s	61.7 s	52.0 s	53.0 s
9	60.6 d	50.1 d	50.2 d	46.1 d	48.8 d	53.0 d
10	38.6 s	38.4 s	38.3 s	37.5 s	39.1 s	39.4 s
11	69.0 d	15.8 t	19.8 t	16.5 t	16.3 t	17.6 t
12	28.3 t	19.2 t	26.1 t	20.6 t	23.1 t	28.1 t
13	33.8 d	39.2 d	35.8 d	38.4 d	37.5 d	41.1 d
14	48.5 d	50.0 d	41.1 d	41.4 d	42.6 d	53.3 d
15	67.6 t	68.2 t	102.6 d	63.0 t	104.0 d	200.4 d
16	180.8 s	175.8 s	179.0 s	175.5 s	176.8 s	173.8 s
17	18.0 q	98.9 d	173.1 d	168.2 d	103.3 d	202.4 d
18	33.5 q	33.7 q	33.0 q	33.1 q	30.5 q	36.6 q
19	21.5 q	21.8 q	21.3 q	21.3 q	16.3 q	22.0 q
20	17.1 q	14.8 q	17.2 q	16.3 q	16.6 q	17.1 q
$OCOCH_3$	170.3 s	170.8 s	170.5 s	170.4 s	171.2 s	
$OCOCH_3$	21.7 q	21.5 q	21.5 q	21.4 q	21.3 q	
$COOCH_3$		51.7 q		51.9 q		52.4 q
$OCOCH_2CH_2CH_3$					36.6 t	36.6 t
$OCOCH_2CH_2CH_3$					18.8 t	18.9 t
$OCOCH_2CH_2CH_3$					13.8 q	13.9 q
$OCOCH_2CH_2CH_3$					174.5 s	173.8 s

[A]Chemical shifts [ppm] referenced to $CDCl_3$ (δ_C 77.16).
[B]Some assignments by HMBC experiments.
[C]Data recorded at 125 MHz.
[D]Data recorded at 188 MHz.
[E]Data recorded at 225 MHz.

Diterpene **3** was obtained as a colourless oil by NP-HPLC (hexanes/EtOAc, 60/40), and had a molecular formula of $C_{22}H_{31}NO_4$ inferred from HR-ESI MS (*m/z* 396.2147 $[M+Na]^+$). The ^1H spectrum (Table 1) revealed three methyl singlets (δ_H 1.03, 0.83, 0.81) and an acetate methyl (δ_H 2.16) signals, which were similar to those found in aplyroseol-2 (**14**),[22] but there was only one acetal proton (δ_H 6.30) observed. A downfield signal (δ_H 7.99) linked to a ^{13}C signal at δ_C 173.1 by HSQC suggested an imine.[32] This was positioned at C17 from HMBC cross-peaks from δ_H 7.99 to C8 (δ_C 63.2), C14 (δ_C 41.1) and the acetal at C15 (δ_C 102.6), and from a COSY correlation between H15 and H17. A 7-OAc group was determined by COSY ($H7/H_26$) and HMBC correlations (H7 to C5 and the acetate carbonyl). The appearance of H13 differed from that in either **1** or **2**; there was clearly a 12.0 Hz coupling between H13 and H14, suggesting they were eclipsed. The *J* value of 9.1 Hz between H13 and H12β was smaller than that observed in either **1** or **2**, but is too large to be anything other than an axial-axial coupling as evidenced by a coupling between H13 and the equatorial H12α of 2.6 Hz. As in **1**, but in contrast to **2**, there was no evidence of an NOE between H9 and H13 for which an inter proton distance of 3.1 Å was measured by modelling. The presentation of the H13 signal was also inconsistent with an alternative conformation, in which ring C adopts a flattened chair shape, for which models revealed that H13 would show similar-sized couplings to each of the H12 protons. The NOEs from H14 to each of H9, H13, and H15, established these protons on the bottom face of the rigid C–D–E ring system, an NOE from H17/Me-20 supported the configuration at C8, and NOEs from H7β to both H17 and H15 confirmed the 7α-OAc.

The molecular formula of diterpene **4** was $C_{23}H_{35}NO_4$ by HR-ESI MS, with the presence of nitrogen again suggesting an imine, as in **3**. The $^1H/^{13}C$ NMR and HMBC data also revealed a carbomethoxy group at C13 (as in **2**) and an acetate group at C7 (as in **3**). Compared with **3**, the acetal proton at C15 was replaced by signals for a diastereotopic methylene (δ_H 4.00, 3.71). H15β showed HMBC correlations to C13 and there were also HMBC correlations from H13 and H14 to C15. The C17 imine at δ_C 168.2 had HMBC correlations with $H_2$15 and H9, while there was also a long range COSY correlation from both H15 signals to H17 at δ_H 7.57. The NOE correlations observed between H13/H9, H13/H14, H7/H14, H7/H17 confirmed the α orientations of H13, H14, and the 7-OAc group. To our knowledge, imine functionality, as seen in **3** and **4**, has not been encountered in this class of compound before. Unfortunately attempts to reduce the imine moiety using H_2/Pd-C gave multiple products that could not be definitively characterized given the small amounts of product available. We named the new diterpenes **3** and **4** as chromoculatimine A and B, respectively.

Diterpene **5** was isolated as a colourless oil that gave a $[M+Na]^+$ ion at *m/z* 517.2397 in the HR-ESI MS, matching the molecular formula $C_{26}H_{38}O_9$. The ^1H and ^{13}C NMR spectra showed evidence for three methyl groups (δ_H 1.10, 1.05, 1.01), a butyrate ester (δ_H 2.45, 1.73, 1.01; δ_C 174.5 s, 36.6 t, 18.8 t,

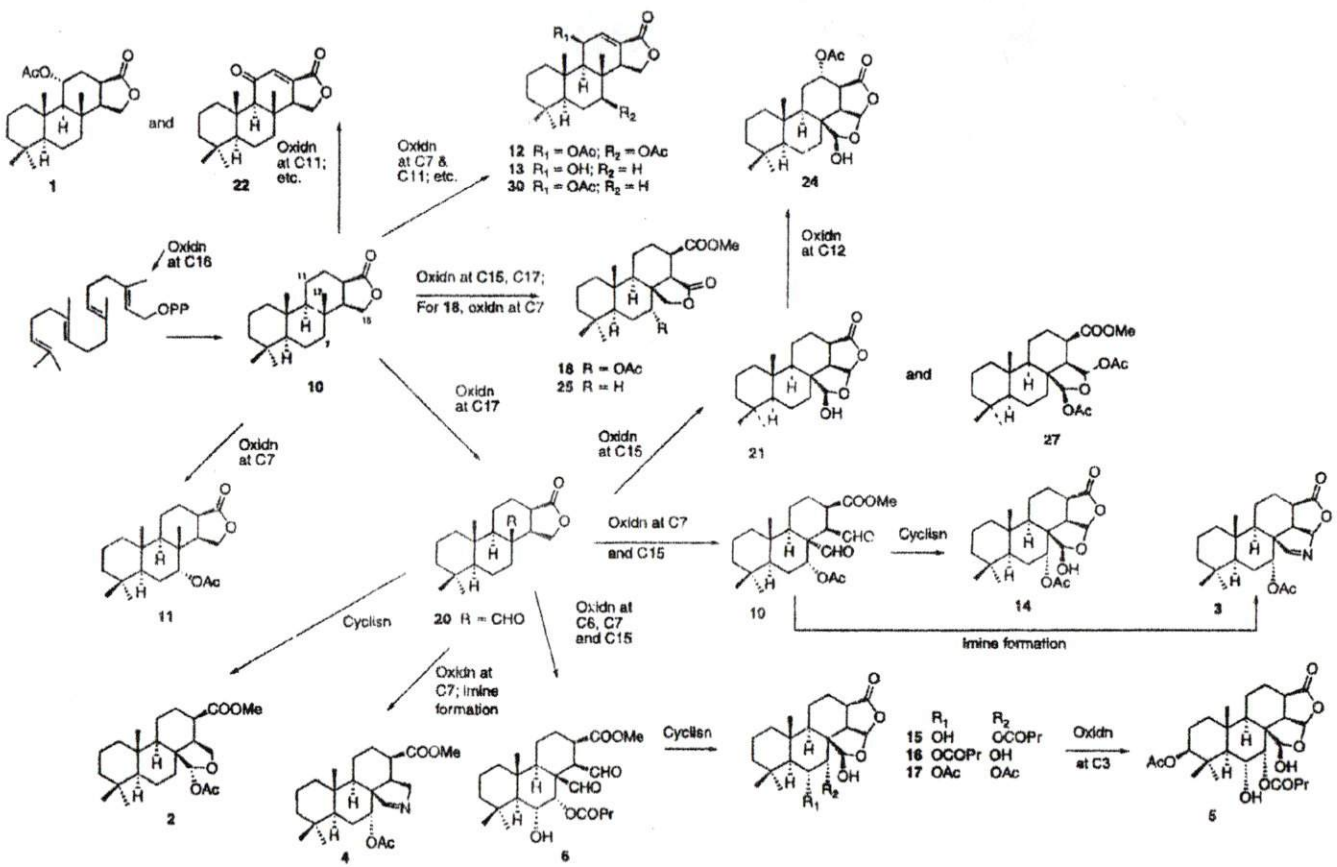

Fig. 3. Putative metabolic transformations in dendroceratid sponges and their mollusc predator *Chromodoris reticulata*.

13.8 q), an acetate methyl (δ_H 2.07, δ_C 21.3), and two acetal groups (δ_H 6.06, 5.53; δ_C 104.0, 103.3). These structural features were similar to those seen in the aplyroseol series of metabolites,[13,22,23,27] notably aplyroseol-3 (**15**),[22] except there was an additional oxymethine signal at δ_H 4.50 for H3 that appeared as a doublet of doublets (J 11.8, 4.8 Hz) revealing the axial position of H3, and hence a β orientation for the C3 substituent, that was further confirmed by NOESY correlations from H3 to H1α, H5, and Me-18. HMBC, COSY, and 1D-TOCSY experiments revealed that the acetate, hydroxyl, and butyrate ester groups were attached to C3, C6, and C7, respectively. The axial H6 at δ_H 4.26 (ddd, J 11.3, 6.0, 2.6 Hz) showed coupling to H5 (δ_H 1.60, d, 11.3), the equatorial H7 (δ_H 4.95, d, 2.6) and an OH (δ_H 1.44, d, 6.0). As in aplyroseol-3 (**15**), NOEs from H17 to Me-20, H6, and H7 confirmed a 6α-OH, a 17β-OH, and an α-orientated butyrate group. Additional NOEs were between H7/H14 and H7/H15.

The HR-ESI MS of diterpene **6** displayed a sodiated molecular ion at *m/z* 473.2508 corresponding to a molecular formula $C_{25}H_{38}O_7$. The ^{1}H and ^{13}C NMR spectra resembled those of the known aldehyde **19**,[20] and included three methyl singlets (δ_H 1.08, 0.95, 0.85), a carboxymethyl (δ_H 3.67; δ_C 52.4), and two aldehyde protons (δ_H 10.00, 9.73; δ_C 202.4, 200.4). However, there was no acetate group present; instead there was a butyrate ester (δ_H 2.38, 1.68, 0.98; δ_C 173.8 s, 36.6 t, 18.9 t, 13.9 q) and a hydroxy group (δ_H 1.56, detected by DQFCOSY). The hydroxy group and butyrate ester were identified as attached to C6 (δ_H 4.09; δ_C 69.3) and C7 (δ_H 6.07; δ_C 73.9), respectively, by HMBC experiments. As in **19**, both aldehydes were β oriented since there were NOESY cross peaks from H15 to H7 and H14, and from H17 to H6, H7, and Me-20. The α orientations of the butyrate ester and the hydroxy group were confirmed by NOEs between H6/Me-20, H6/Me-19, H6/H17, H6/H7, H7/H15, and H7/H17.

Consistent $[\alpha]_D$ trends in this series of highly functionalized diterpenes are not readily apparent. In our earlier work, metabolite **24** was assigned to the same enantiomeric series as (+)-isoagatholactone (**28**),[33] for which the absolute configuration has been established based on total synthesis from (+)-manool. In contrast, aplyroseol-1 (**29**), with the same absolute configuration deduced from an X-ray study, has a negative $[\alpha]_D$ value.[34]

Following the careful dissection and analysis of the second *Chromodoris* specimen, ^{1}H NMR spectra of extracts or fractions obtained from the mantle and internal organs were compared. The mantle tissue extract contained diterpenes **1–6**, **9**, **11–14**, **16**, **17**, and **20–22**, while ambliofuran **7** was found only in the internal organs. The remaining diterpenes **8**, **10**, **15**, **18**, **19**, and **23** were found in both tissue types. Aplysulfurin (**8**) was the major component in the internal organs, while aplyroseol-2 (**14**) was the major compound in the mantle tissue. As in our previous study,[20] dialdehydes such as **19** were concentrated in the mantle tissue compared with the internal organs.

Based on the pattern of metabolites from this particular nudibranch, the dietary origin of these oxidised diterpenes could be dendroceratid sponges.[35] As noted earlier, dialdehyde **19** provides acetal **14** directly on cyclization (Fig. 3);[20] consequently new dialdehyde **6** could be the actual source of acetal **15**. Imine **3** may result from formal addition of ammonia (or equivalent) to dialdehyde **19**, while the absence of aldehyde functionality at C15 in e.g. **20** limits additional cyclization and leads to diterpene **2** or imine **4**, each with a free carbomethoxy group. In contrast, lactone **18** apparently results from oxidation of a dialdehyde at C15 and reduction at C17, or alternatively is the eventual product of incomplete oxidation of the C17 Me group in an earlier intermediate on the biosynthetic pathway. Lactones **1** and **11** may each derive from oxidation and acetylation of lactone **10**, reactions which by analogy with the established chemistry of various *Glossodoris* molluscs[36] might be expected to occur in the mollusc rather than the dietary source. However a related profile of oxidation-acetylation is also seen in lactones **12** and **13**, structurally related to the known sponge compound **30**.

Conclusion

This study reported six new diterpenes (**1–6**) along with 17 known metabolites previously isolated from dendroceratid sponges and from the nudibranch *C. obsoleta*. Fifteen of these diterpenes were present only in mantle tissue, while seven of the remaining eight metabolites were present in both mantle and digestive tissues extracts. The linear furan ambliofuran **7** was the only diterpene found solely in the internal organs extract. The presence of lactone-acetal-hemiacetal functionality in many of the isolated diterpenes is likely related to dialdehyde precursors.

Experimental

General

Optical rotations were obtained using a Perkin–Elmer 241-MC polarimeter. 1D and 2D NMR spectra were acquired using Bruker Avance 500, 750, and 900 instruments. NMR spectra were obtained in deuterochloroform or benzene-d_6 at room temperature, and were internally referenced to $CHCl_3$ (δ_H 7.26) or C_6H_6 (δ_H 7.16) and $CDCl_3$ (δ_C 77.16) or C_6D_6 (δ_C 128.06). Positive ion electrospray mass spectra (LR-ES MS) were determined using a Bruker Esquire HCT instrument or (HR-ESI MS) using a MicroTof Q instrument each with a standard ESI source. Samples were introduced into the source using MeOH as solvent. NP-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, flow rate 2 mL min^{-1}. 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), using MeOH/H_2O as a solvent at flow rate 1.5 mL min^{-1}.

Collection, Extraction, and Isolation

Two mollusc specimens were collected from Hanging Rock dive site, at the Inner Gneerings reef, a group of shoals near Mooloolaba (Australia), using SCUBA at a depth of 10–15 m on 5 December 2009. Subsequent examination revealed their identity was *Chromodoris reticulata*.[37] The samples were taken back to the laboratory and stored at −20°C until extraction.

A specimen of *Chromodoris reticulata* (wet weight 9.4 g) was diced and extracted exhaustively with acetone by using ultrasonic vibration for 30 min. The extract was removed, filtered through cotton, and concentrated under reduced pressure to give an aqueous residue, which was then partitioned with EtOAc. The organic layer was removed, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to give a brown crude extract (238 mg). The extract was then subjected to SiO_2 flash chromatography with gradient elution (hexanes to EtOAc) to give 13 fractions. Compounds **7** (0.9 mg), **10** (16.4 mg), and

14 (74.2 mg) were identified in fractions 2, 6, and 10, respectively. Fraction 7 (31.4 mg) was subjected to NP-HPLC (hexanes/EtOAc, 80/20) to afford the new compound **2** (1.5 mg) and diterpene **8** (6.6 mg). Fraction 8 (10.5 mg) was subjected to reverse phase-HPLC using gradient elution of MeOH/H_2O (70/30) to 100% MeOH for 40 min, and gave compounds **9** (2.3 mg) and **11** (1.5 mg). A fraction from the reverse phase-HPLC containing a mixture of compounds was subjected to NP-HPLC (hexanes/EtOAc, 75/25), and gave a mixture of compounds **13** and **21** (1.8 mg), the new lactone **1** (<0.3 mg), and a mixture of lactone **11** and aldehyde **20** (1.3 mg). Fraction 9 (12 mg) was chromatographed on NP-HPLC (hexanes/EtOAc, 80/20) to afford compounds **12** (0.4 mg) and **18** (0.3 mg). Fractions 11 and 12 were combined (19.7 mg), and chromatographed on NP-HPLC (hexanes/EtOAc, 60/40) to give aldehyde **19** (1.9 mg), a mixture of compounds **14** and **15** (6.3 mg), as well as compounds **17** (1.4 mg), **16** (3.1 mg), and the new imine **3** (1.8 mg), in order of elution.

The second specimen of *Chromodoris reticulata* (wet weight 7.8 g) was dissected into mantle (wet weight 3.9 g) and internal organs (wet weight 2.8 g). Each section was extracted using the same procedure as for the first animal, to give an orange oil (232 mg) from the mantle and a yellow oily extract (66 mg) from the internal organs. The mantle extract was subjected to SiO_2 flash chromatography with gradient elution (hexanes to EtOAc) to give 14 fractions. Fraction 9 (15.0 mg, hexanes/EtOAc, 80/20) was subjected to NP-HPLC (hexanes/EtOAc, 80/20) to give lactone **22** (0.3 mg). Fraction 12 (37.0 mg, hexanes/EtOAc, 65/35) was subjected to NP-HPLC (hexanes/EtOAc, 60/40) to afford compound **23** (1.7 mg). Fractions 7 and 8 from the NP-HPLC of fraction 12 were combined before further NP-HPLC (hexanes/EtOAc, 70/30) to give dialdehyde **6** (~0.2 mg). Fraction 13 (12.8 mg, hexanes/EtOAc, 60/40) was also subjected to the same NP-HPLC conditions to give diterpene **5** (0.5 mg), imine **3** (0.9 mg), and imine **4** (0.4 mg), in order of elution.

11α-Acetoxy-spongian-16-one (1)

Colourless oil; $[\alpha]_D$ +73 (*c* 0.01 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) and δ_C ($CDCl_3$, 125 MHz), see Tables 1 and 2; δ_H (benzene-d_6, 500 MHz) 5.17 (1H, td, *J* 5.0, 2.0 Hz, H11), 3.73 (1H, dd, *J* 10.0, 2.1 Hz, H15β), 3.56 (1H, dd, *J* 10.0, 8.1 Hz, H15α), 2.35 (1H, td, *J* 11.0, 8.8 Hz, H13), 2.23 (1H, ddd, *J* 16.0, 8.8, 2.1 Hz, H12α), 1.89 (1H, ddd, *J* 11.0, 4.8 Hz, H12β), 1.70 (3H, s, OCO*CH*$_3$), 1.51 (1H, m, H14), 1.48 (1H, m, H1β), 1.46 (1H, m, H2α), 1.29 (2H, m, H7β, H2β), 1.25 (1H, m, H6α), 1.09 (1H, dt, *J* 12.5, 3.5 Hz, H3β), 1.06 (1H, m, H7α), 1.01 (1H, m, H6β), 0.88 (1H, m, H1α), 0.84 (1H, d, *J* 5.0 Hz, H9), 0.80 (3H, s, Me-18), 0.71 (3H, s, Me-19), 0.60 (1H, dd, *J* 12.4, 2.5 Hz, H5), 0.60 (3H, s, Me-20), 0.49 (3H, s, Me-17), 0.41 (1H, m, H3α), assignment of H2 and H6 may be interchanged, δ_C (benzene-d_6, 125 MHz, partial data from HSQC) 68.9 (C11), 66.5 (C15), 60.5 (C9), 56.8 (C5), 48.3 (C14), 42.2 (C7), 41.6 (C3), 38.6 (C1), 33.4 (Me-18), 28.4 (C12), 21.3 (Me-19), 21.1 (OCO*CH*$_3$), 18.3 (C2), 18.0 (C6), 17.4 (Me-17), 16.7 (Me-20), assignment of C2 and C6 may interchanged; HR-ESI MS *m/z* 385.2345 $[M+Na]^+$; calc. for $C_{22}H_{34}O_4Na$: 385.2349.

Methyl 15,17-Epoxy-17α-acetoxy-ent-isocopalan-16-oate (2)

Colourless oil; $[\alpha]_D$ +31 (*c* 0.10 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) and δ_C ($CDCl_3$, 125 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 415.2463 $[M+Na]^+$; calc. for $C_{23}H_{36}O_5Na$: 415.2455.

Chromoculatimine A (3)

Colourless oil; $[\alpha]_D$ −13 (*c* 0.09 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 396.2147 $[M+Na]^+$; calc. for $C_{22}H_{31}NO_4Na$: 396.2145.

Chromoculatimine B (4)

Colourless oil; $[\alpha]_D$ −24 (*c* 0.03 in $CHCl_3$); δ_H ($CDCl_3$, 750 MHz) and δ_C ($CDCl_3$, 188 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 412.2467 $[M+Na]^+$; calc. for $C_{23}H_{35}NO_4Na$: 412.2458.

Aplyroseol-19 (5)

Colourless oil; $[\alpha]_D$ +9 (*c* 0.03 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) and δ_C ($CDCl_3$, 125 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 517.2397 $[M+Na]^+$; calc. for $C_{26}H_{38}O_9Na$: 517.2408.

Methyl 6α-Hydroxy-7α-butyryloxy-8β,14β-diformyipodocarpane-13β-carboxylate (6)

Colourless oil; $[\alpha]_D$ −19 (*c* 0.01 in $CHCl_3$); δ_H ($CDCl_3$, 900 MHz) and δ_C ($CDCl_3$, 225 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 473.2508 $[M+Na]^+$; calc. for $C_{25}H_{38}O_7Na$: 473.2510.

Spongi-12-en-11,16-dione (22)

Colourless oil; $[\alpha]_D$ +44 (*c* 0.02 in $CHCl_3$), lit. $[\alpha]_D$ +34.5 (*c* 1.08 in $CHCl_3$);[26] δ_H ($CDCl_3$, 750 MHz) 6.54 (1H, d, *J* 3.5 Hz, H12), 4.54 (1H, t, *J* 9.2 Hz, H15β), 4.16 (1H, t, *J* 9.2 Hz, H15α), 3.29 (1H, td, *J* 9.2, 3.9 Hz, H14), 2.59 (1H, dq, *J* 12.8, 3.1 Hz, H1β), 2.11 (1H, s, H9), 1.72 (1H, dq, *J* 12.7, 3.1 Hz, H7β), 1.66 (1H, td, *J* 13.7, 3.5 Hz, H2α), 1.62 (1H, m, H6α), 1.55 (1H, m, H7α), 1.42–1.41 (3H, m, H2β, H6β, H3β), 1.17 (1H, td, *J* 13.4, 4.1 Hz, H3α), 1.15 (3H, s, Me-20), 0.96 (3H, s, Me-17), 0.88 (3H, s, Me-18), 0.86 (1H, dd, *J* 12.4, 2.1 Hz, H5), 0.85 (3H, s, Me-19), 0.81 (1H, m, H1α); δ_C ($CDCl_3$, 188 MHz) 199.4 (C11), 168.8 (C16), 142.6 (C13), 130.4 (C12), 69.1 (C9), 67.1 (C15), 56.4 (C5), 52.6 (C14), 44.0 (C8), 42.1 (C3), 41.3 (C7), 39.7 (C1), 37.8 (C10), 33.7 (C4 and Me-18), 21.9 (Me-19), 18.3 (C2), 17.7 (C6), 16.2 (Me-20), 15.7 (Me-17); HR-ESI MS *m/z* 339.1927 $[M+Na]^+$; calc. for $C_{20}H_{28}O_3Na$: 339.1931.

Accessory Publication

The Accessory Publication contains copies of representative NMR spectra, including the ^1H spectra of metabolites (**1**–**6**) in $CDCl_3$, together with HMBC and NOESY data. The Accessory Publication is available on the Journal's website.

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Structures and Anatomical Distribution of Oxygenated Diterpenes in the Australian Nudibranch Chromodoris reticulata

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Table 2. ^{13}C NMR data for diterpenes **1**–**6**[A,B]

C	1 δ_C[C]	2 δ_C[C]	3 δ_C[C]	4 δ_C[D]	5 δ_C[C]	6 δ_C[E]
1	39.5t	39.6t	38.9t	38.9t	37.1t	39.1t
2	18.2t	18.6t	18.5t	18.4t	23.5t	18.7t
3	42.1t	42.1t	41.9t	42.0t	80.3d	43.4t
4	33.5s	33.6s	33.0s	32.8s	37.8s	33.4s
5	56.9d	56.9d	48.0d	48.7d	52.5d	52.0d
6	18.2t	20.7t	25.3t	25.6t	69.9d	69.3d
7	42.2t	37.4t	73.8d	75.2d	77.0d	73.9d
8	35.7s	49.6s	63.2s	61.7s	52.0s	53.0s
9	60.6d	50.1d	50.2d	46.1d	48.8d	53.0d
10	38.6s	38.4s	38.2s	37.5s	39.1s	39.4s
11	69.0d	15.8t	19.8t	16.5t	16.3t	17.6t
12	28.3t	19.2t	26.1t	20.6t	23.1t	28.1t
13	33.8d	39.2d	35.8d	38.4d	37.5d	41.1d
14	48.5d	50.0d	41.1d	41.4d	42.6d	53.3d
15	67.6t	68.2t	102.6d	63.0t	104.0d	200.4d
16	180.8s	175.8s	179.0s	175.5s	176.8s	173.8s
17	18.0q	98.9d	173.1d	168.2d	103.3d	202.4d
18	33.5q	33.7q	33.0q	33.1q	30.5q	36.6q
19	21.5q	21.8q	21.3q	21.3q	16.3q	22.0q
20	17.1q	14.8q	17.2q	16.3q	16.6q	17.1q
$OCOCH_3$	170.3s	170.8s	170.5s	170.4s	171.2s	
$OCOCH_3$	21.7q	21.5q	21.5q	21.4q	21.3q	
$COOCH_3$		51.7q		51.9q		52.4q
$OCOCH_2CH_2CH_3$					36.6t	36.6t
$OCOCH_2CH_2CH_3$					18.8t	18.9t
$OCOCH_2CH_2CH_3$					13.8q	13.9q
$OCOCH_2CH_2CH_3$					174.5s	173.8s

[A]Chemical shifts [ppm] referenced to $CDCl_3$ (δ_C 77.16).
[B]Some assignments by HMBC experiments.
[C]Data recorded at 125 MHz.
[D]Data recorded at 188 MHz.
[E]Data recorded at 225 MHz.

Diterpene **3** was obtained as a colourless oil by NP-HPLC (hexanes/EtOAc, 60/40), and had a molecular formula of $C_{22}H_{31}NO_4$ inferred from HR-ESI MS (*m/z* 396.2147 $[M+Na]^+$). The ^1H spectrum (Table 1) revealed three methyl singlets (δ_H 1.03, 0.83, 0.81) and an acetate methyl (δ_H 2.16) signals, which were similar to those found in aplyroseol-2 (**14**),[122] but there was only one acetal proton (δ_H 6.30) observed. A downfield signal (δ_H 7.99) linked to a ^{13}C signal at δ_C 173.1 by HSQC suggested an imine.[124] This was positioned at C17 from HMBC cross-peaks from δ_H 7.99 to C8 (δ_C 63.2), C14 (δ_C 41.1) and the acetal at C15 (δ_C 102.6), and from a COSY correlation between H15 and H17. A 7-OAc group was determined by COSY ($H7/H_26$) and HMBC correlations (H7 to C5 and the acetate carbonyl). The appearance of H13 differed from that in either **1** or **2**; there was clearly a 12.0 Hz coupling between H13 and H14, suggesting they were eclipsed. The *J* value of 9.1 Hz between H13 and H12β was smaller than that observed in either **1** or **2**, but is too large to be anything other than an axial-axial coupling as evidenced by a coupling between H13 and the equatorial H12α of 2.6 Hz. As in **1**, but in contrast to **2**, there was no evidence of an NOE between H9 and H13 for which an inter proton distance of 3.1 Å was measured by modelling. The presentation of the H13 signal was also inconsistent with an alternative conformation, in which ring C adopts a flattened chair shape, for which models revealed that H13 would show similar-sized couplings to each of the H12 protons. The NOEs from H14 to each of H9, H13, and H15, established these protons on the bottom face of the rigid C–D–E ring system, an NOE from H17/Me-20 supported the configuration at C8, and NOEs from H7β to both H17 and H15 confirmed the 7α-OAc.

The molecular formula of diterpene **4** was $C_{23}H_{35}NO_4$ by HR-ESI MS, with the presence of nitrogen again suggesting an imine, as in **3**. The $^1H/^{13}C$ NMR and HMBC data also revealed a carbomethoxy group at C13 (as in **2**) and an acetate group at C7 (as in **3**). Compared with **3**, the acetal proton at C15 was replaced by signals for a diastereotopic methylene (δ_H 4.00, 3.71). H15β showed HMBC correlations to C13 and there were also HMBC correlations from H13 and H14 to C15. The C17 imine at δ_C 168.2 had HMBC correlations with H_215 and H9, while there was also a long range COSY correlation from both H15 signals to H17 at δ_H 7.57. The NOE correlations observed between H13/H9, H13/H14, H7/H14, H7/H17 confirmed the α orientations of H13, H14, and the 7-OAc group. To our knowledge, imine functionality, as seen in **3** and **4**, has not been encountered in this class of compound before. Unfortunately attempts to reduce the imine moiety using H_2/Pd-C gave multiple products that could not be definitively characterized given the small amounts of product available. We named the new diterpenes **3** and **4** as chromoculatimine A and B, respectively.

Diterpene **5** was isolated as a colourless oil that gave a $[M+Na]^+$ ion at *m/z* 517.2397 in the HR-ESI MS, matching the molecular formula $C_{28}H_{38}O_9$. The ^1H and ^{13}C NMR spectra showed evidence for three methyl groups (δ_H 1.10, 1.05, 1.01), a butyrate ester (δ_H 2.45, 1.73, 1.01; δ_C 174.5 s, 36.6 t, 18.8 t,

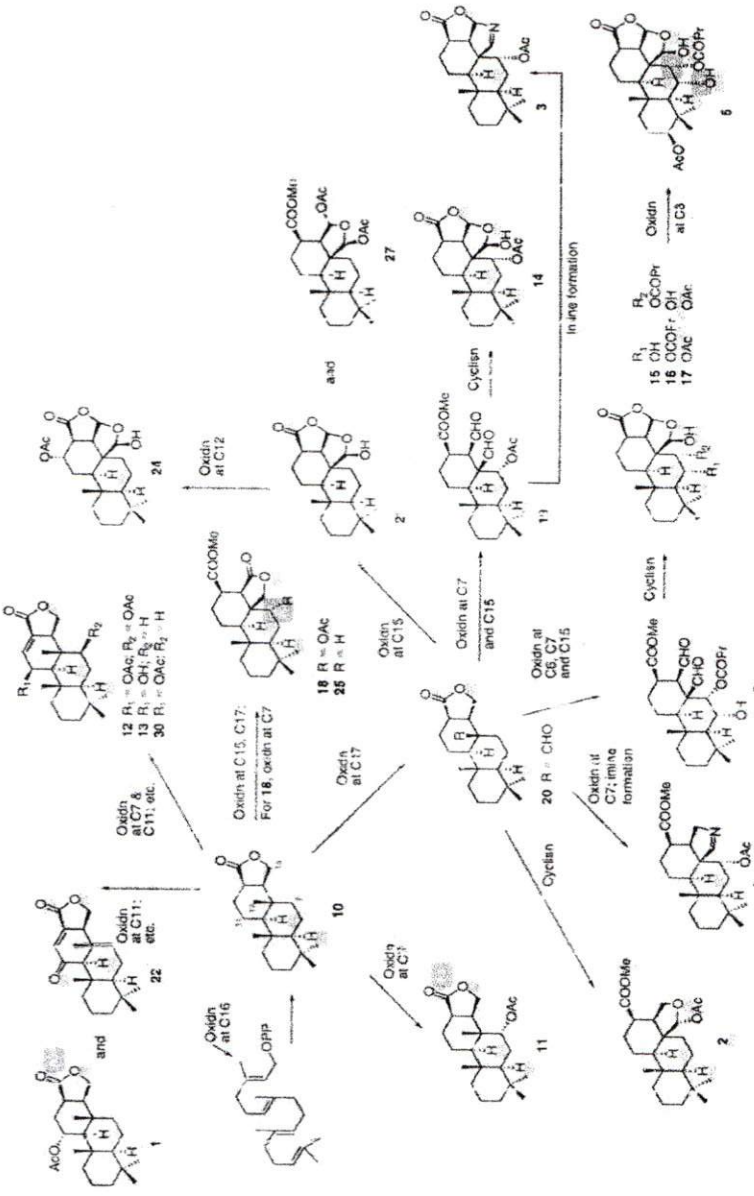

Fig. 3. Putative metabolic transformations in dendroceratid sponges and their mollusc predator *Chromodoris reticulata*.

13.8 q), an acetate methyl (δ_H 2.07, δ_C 21.3), and two acetal groups (δ_H 6.06, 5.53; δ_C 104.0, 103.3). These structural features were similar to those seen in the aplyroseol series of metabolites,[13,22,23,27] notably aplyroseol-3 (**15**),[22] except there was an additional oxymethine signal at δ_H 4.50 for H3 that appeared as a doublet of doublets (J 11.8, 4.8 Hz) revealing the axial position of H3, and hence a β orientation for the C3 substituent, that was further confirmed by NOESY correlations from H3 to H1α, H5, and Me-18. HMBC, COSY, and 1D-TOCSY experiments revealed that the acetate, hydroxyl, and butyrate ester groups were attached to C3, C6, and C7, respectively. The axial H6 at δ_H 4.26 (ddd, J 11.3, 6.0, 2.6 Hz) showed coupling to H5 (δ_H 1.60, d, 11.3), the equatorial H7 (δ_H 4.95, d, 2.6) and an OH (δ_H 1.44, d, 6.0). As in aplyroseol-3 (**15**), NOEs from H17 to Me-20, H6, and H7 confirmed a 6α-OH, a 17β-OH, and an α-orientated butyrate group. Additional NOEs were between H7/H14 and H7/H15.

The HR-ESI MS of diterpene **6** displayed a sodiated molecular ion at m/z 473.2508 corresponding to a molecular formula $C_{25}H_{38}O_7$. The ^{1}H and ^{13}C NMR spectra resembled those of the known aldehyde **19**,[20] and included three methyl singlets (δ_H 1.08, 0.95, 0.85), a carboxymethyl (δ_H 3.67; δ_C 52.4), and two aldehyde protons (δ_H 10.00, 9.73; δ_C 202.4, 200.4). However, there was no acetate group present; instead there was a butyrate ester (δ_H 2.38, 1.68, 0.98; δ_C 173.8 s, 36.6 t, 18.9 t, 13.9 q) and a hydroxy group (δ_H 1.56, detected by DQFCOSY). The hydroxy group and butyrate ester were identified as attached to C6 (δ_H 4.09, δ_C 69.3) and C7 (δ_H 6.07, δ_C 75.9), respectively, by HMBC experiments. As in **19**, both aldehydes were β oriented since there were NOESY cross peaks from H15 to H7 and H14, and from H17 to H6, H7, and Me-20. The α orientations of the butyrate ester and the hydroxy group were confirmed by NOEs between H6/Me-20, H6/Me-19, H6/H17, H6/H7, H7/H15, and H7/H17.

Consistent $[\alpha]_D$ trends in this series of highly functionalized diterpenes are not readily apparent. In our earlier work, metabolite **24** was assigned to the same enantiomeric series as (+)-isoagatholactone (**28**),[33] for which the absolute configuration has been established based on total synthesis from (+)-manool. In contrast, aplyroseol-1 (**29**), with the same absolute configuration deduced from an X-ray study, has a negative $[\alpha]_D$ value.[34]

Following the careful dissection and analysis of the second *Chromodoris* specimen, ^{1}H NMR spectra of extracts or fractions obtained from the mantle and internal organs were compared. The mantle tissue extract contained diterpenes **1**–**6**, **9**, **11**–**14**, **16**, **17**, and **20**–**22**, while ambliofuran **7** was found only in the internal organs. The remaining diterpenes **8**, **10**, **15**, **18**, **19**, and **23** were found in both tissue types. Aplysulfurin (**8**) was the major component in the internal organs, while aplyroseol-2 (**14**) was the major compound in the mantle tissue. As in our previous study,[20] dialdehydes such as **19** were concentrated in the mantle tissue compared with the internal organs.

Based on the pattern of metabolites from this particular nudibranch, the dietary origin of these oxidised diterpenes could be dendroceratid sponges.[35] As noted earlier, dialdehyde **19** provides acetal **14** directly on cyclization (Fig. 3);[20] consequently new dialdehyde **6** could be the actual source of acetal **15**. Imine **3** may result from formal addition of ammonia (or equivalent) to dialdehyde **19**, while the absence of aldehyde functionality at C15 in e.g. **20** limits additional cyclization and leads to diterpene **2** or imine **4**, each with a free carbomethoxy group. In contrast, lactone **18** apparently results from oxidation of a dialdehyde at C15 and reduction at C17, or alternatively is the eventual product of incomplete oxidation of the C17 Me group in an earlier intermediate on the biosynthetic pathway. Lactones **1** and **11** may each derive from oxidation and acetylation of lactone **10**, reactions which by analogy with the established chemistry of various *Glossodoris* molluscs[36] might be expected to occur in the mollusc rather than the dietary source. However a related profile of oxidation-acetylation is also seen in lactones **12** and **13**, structurally related to the known sponge compound **30**.

Conclusion

This study reported six new diterpenes (**1**–**6**) along with 17 known metabolites previously isolated from dendroceratid sponges and from the nudibranch *C. obsoleta*. Fifteen of these diterpenes were present only in mantle tissue, while seven of the remaining eight metabolites were present in both mantle and digestive tissues extracts. The linear furan ambliofuran **7** was the only diterpene found solely in the internal organs extract. The presence of lactone-acetal-hemiacetal functionality in many of the isolated diterpenes is likely related to dialdehyde precursors.

Experimental

General

Optical rotations were obtained using a Perkin–Elmer 241-MC polarimeter. 1D and 2D NMR spectra were acquired using Bruker Avance 500, 750, and 900 instruments. NMR spectra were obtained in deuterochloroform or benzene-d_6 at room temperature, and were internally referenced to $CHCl_3$ (δ_H 7.26) or C_6H_6 (δ_H 7.16) and $CDCl_3$ (δ_C 77.16) or C_6D_6 (δ_C 128.06). Positive ion electrospray mass spectra (LR-ES MS) were determined using a Bruker Esquire HCT instrument or (HR-ESI MS) using a MicroTof Q instrument each with a standard ESI source. Samples were introduced into the source using MeOH as solvent. NP HPLC was carried out using a Waters 515 pump with a Waters 10μ μPerasil 7.8 × 300 mm column and a Gilson 132 series RI detector with EtOAc/hexanes as solvent, flow rate 2 mL min^{-1}. 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), using MeOH/H_2O as a solvent at flow rate 1.5 mL min^{-1}.

Collection, Extraction, and Isolation

Two mollusc specimens were collected from Hanging Rock dive site, at the Inner Gneerings reef, a group of shoals near Mooloolaba (Australia), using SCUBA at a depth of 10–15 m on 5 December 2009. Subsequent examination revealed their identity was *Chromodoris reticulata*.[37] The samples were taken back to the laboratory and stored at −20°C until extraction.

A specimen of *Chromodoris reticulata* (wet weight 9.4 g) was diced and extracted exhaustively with acetone by using ultrasonic vibration for 30 min. The extract was removed, filtered through cotton, and concentrated under reduced pressure to give an aqueous residue, which was then partitioned with EtOAc. The organic layer was removed, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to give a brown crude extract (228 mg). The extract was then subjected to SiO_2 flash chromatography with gradient elution (hexanes to EtOAc) to give 13 fractions. Compounds **7** (0.9 mg), **10** (16.4 mg), and

14 (74.2 mg) were identified in fractions 2, 6, and 10, respectively. Fraction 7 (31.4 mg) was subjected to NP-HPLC (hexanes/EtOAc, 80/20) to afford the new compound **2** (1.5 mg) and diterpene **8** (6.6 mg). Fraction 8 (10.5 mg) was subjected to reverse phase-HPLC using gradient elution of MeOH/H_2O (70/30) to 100% MeOH for 40 min, and gave compounds **9** (2.3 mg) and **11** (1.5 mg). A fraction from the reverse phase-HPLC containing a mixture of compounds was subjected to NP-HPLC (hexanes/EtOAc, 75/25), and gave a mixture of compounds **13** and **21** (1.8 mg), the new lactone **1** (<0.3 mg), and a mixture of lactone **11** and aldehyde **20** (1.3 mg). Fraction 9 (12 mg) was chromatographed on NP-HPLC (hexanes/EtOAc, 80/20) to afford compounds **12** (0.4 mg) and **18** (0.3 mg). Fractions 11 and 12 were combined (19.7 mg), and chromatographed on NP-HPLC (hexanes/EtOAc, 60/40) to give aldehyde **19** (1.9 mg), a mixture of compounds **14** and **15** (6.3 mg), as well as compounds **17** (1.4 mg), **16** (3.1 mg), and the new imine **3** (1.8 mg), in order of elution.

The second specimen of *Chromodoris reticulata* (wet weight 7.8 g) was dissected into mantle (wet weight 3.9 g) and internal organs (wet weight 2.8 g). Each section was extracted using the same procedure as for the first animal, to give an orange oil (232 mg) from the mantle and a yellow oily extract (66 mg) from the internal organs. The mantle extract was subjected to SiO_2 flash chromatography with gradient elution (hexanes to EtOAc) to give 14 fractions. Fraction 9 (15.0 mg, hexanes/EtOAc, 80/20) was subjected to NP-HPLC (hexanes/EtOAc, 80/20) to give lactone **22** (0.3 mg). Fraction 12 (37.0 mg, hexanes/EtOAc, 65/35) was subjected to NP-HPLC (hexanes/EtOAc, 60/40) to afford compound **23** (1.7 mg). Fractions 7 and 8 from the NP-HPLC of fraction 12 were combined before further NP-HPLC (hexanes/EtOAc, 70/30) to give dialdehyde **6** (~0.2 mg). Fraction 13 (12.8 mg, hexanes/EtOAc, 60/40) was also subjected to the same NP-HPLC conditions to give diterpene **5** (0.5 mg), imine **3** (0.9 mg), and imine **4** (0.4 mg), in order of elution.

11α-Acetoxy-spongian-16-one (1)

Colourless oil; $[\alpha]_D$ +73 (*c* 0.01 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) and δ_C ($CDCl_3$, 125 MHz), see Tables 1 and 2; δ_H (benzene-d_6, 500 MHz) 5.17 (1H, td, *J* 5.0, 2.0 Hz, H11), 3.73 (1H, dd, *J* 10.0, 2.1 Hz, H15β), 3.56 (1H, dd, *J* 10.0, 8.1 Hz, H15α), 2.35 (1H, td, *J* 11.0, 8.8 Hz, H13), 2.23 (1H, ddd, *J* 16.0, 8.8, 2.1 Hz, H12α), 1.89 (1H, ddd, *J* 11.0, 4.8 Hz, H12β), 1.70 (3H, s, $OCOCH_3$), 1.51 (1H, m, H14), 1.48 (1H, m, H1β), 1.46 (1H, m, H2α), 1.29 (2H, m, H7β, H2β), 1.25 (1H, m, H6α), 1.09 (1H, dt, *J* 12.5, 3.5 Hz, H3β), 1.06 (1H, m, H7α), 1.01 (1H, m, H6β), 0.88 (1H, m, H1α), 0.84 (1H, d, *J* 5.0 Hz, H9), 0.80 (3H, s, Me-18), 0.71 (3H, s, Me-19), 0.60 (1H, dd, *J* 12.4, 2.5 Hz, H5), 0.60 (3H, s, Me-20), 0.49 (3H, s, Me-17), 0.41 (1H, m, H3α), assignment of H2 and H6 may be interchanged, δ_C (benzene-d_6, 125 MHz, partial data from HSQC) 68.9 (C11), 66.5 (C15), 60.5 (C9), 56.8 (C5), 48.3 (C14), 42.2 (C7), 41.6 (C3), 38.6 (C1), 33.4 (Me-18), 28.4 (C12), 21.3 (Me-19), 21.1 ($OCOCH_3$), 18.3 (C2), 18.0 (C6), 17.4 (Me-17), 16.7 (Me-20), assignment of C2 and C6 may interchanged; HR-ESI MS *m/z* 385.2345 $[M+Na]^+$; calc. for $C_{22}H_{34}O_4Na$: 385.2349.

Methyl 15,17-Epoxy-17α-acetoxy-ent-isocopalan-16-oate (2)

Colourless oil; $[\alpha]_D$ +31 (*c* 0.10 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) and δ_C ($CDCl_3$, 125 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 415.2463 $[M+Na]^+$; calc. for $C_{23}H_{36}O_5Na$: 415.2455.

Chromoculatimine A (3)

Colourless oil; $[\alpha]_D$ −13 (*c* 0.09 in $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 396.2147 $[M+Na]^+$; calc. for $C_{22}H_{31}NO_4Na$: 396.2145.

Chromoculatimine B (4)

Colourless oil; $[\alpha]_D$ −24 (*c* 0.03 in $CHCl_3$); δ_H ($CDCl_3$, 750 MHz) and δ_C ($CDCl_3$, 188 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 412.2467 $[M+Na]^+$; calc. for $C_{23}H_{35}NO_4Na$: 412.2458.

Aplyroseol-19 (5)

Colourless oil; $[\alpha]_D$ +9 (*c* 0.03 in $CHCl_3$); δ_H ($CDCl_3$, 500 MHz) and δ_C ($CDCl_3$, 125 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 517.2397 $[M+Na]^+$; calc. for $C_{26}H_{38}O_9Na$: 517.2408.

Methyl 6α-Hydroxy-7α-butyryloxy-8β,14β-diformylpodocarpane-13β-carboxylate (6)

Colourless oil; $[\alpha]_D$ −19 (*c* 0.01 in $CHCl_3$); δ_H ($CDCl_3$, 900 MHz) and δ_C ($CDCl_3$, 225 MHz), see Tables 1 and 2; HR-ESI MS *m/z* 473.2508 $[M+Na]^+$; calc. for $C_{25}H_{38}O_7Na$: 473.2510.

Spongi-12-en-11,16-dione (22)

Colourless oil; $[\alpha]_D$ +44 (*c* 0.02 in $CHCl_3$), lit. $[\alpha]_D$ +34.5 (*c* 1.08 in $CHCl_3$);[26] δ_H ($CDCl_3$, 750 MHz) 6.54 (1H, d, *J* 3.5 Hz, H12), 4.54 (1H, t, *J* 9.2 Hz, H15β), 4.16 (1H, t, *J* 9.2 Hz, H15α), 3.29 (1H, td, *J* 9.2, 3.9 Hz, H14), 2.59 (1H, dq, *J* 12.8, 3.1 Hz, H1β), 2.11 (1H, s, H9), 1.72 (1H, dq, *J* 12.8, 3.1 Hz, H7β), 1.66 (1H, td, *J* 13.7, 3.5 Hz, H2α), 1.62 (1H, m, H6α), 1.55 (1H, m, H7α), 1.42–1.41 (3H, m, H2β, H6β, H3β), 1.17 (1H, td, *J* 13.4, 4.1 Hz, H3α), 1.15 (3H, s, Me-20), 0.96 (3H, s, Me-17), 0.88 (3H, s, Me-18), 0.86 (1H, dd, *J* 12.4, 2.1 Hz, H5), 0.85 (3H, s, Me-19), 0.81 (1H, m, H1α); δ_C ($CDCl_3$, 188 MHz) 199.4 (C11), 168.8 (C16), 142.6 (C13), 130.4 (C12), 69.1 (C9), 67.1 (C15), 56.4 (C5), 52.6 (C14), 44.0 (C8), 42.1 (C3), 41.3 (C7), 39.7 (C1), 37.8 (C10), 33.7 (C4 and Me-18), 21.2 (Me-19), 18.3 (C2), 17.7 (C6), 16.2 (Me-20), 15.7 (Me-17); HR-ESI MS *m/z* 339.1927 $[M+Na]^+$; calc. for $C_{20}H_{28}O_3Na$: 339.1931.

Accessory Publication

The Accessory Publication contains copies of representative NMR spectra, including the ^1H spectra of metabolites (**1**–**6**) in $CDCl_3$, together with HMBC and NOESY data. The Accessory Publication is available on the Journal's website.

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Structures and Anatomical Distribution of Oxygenated Diterpenes in the Australian Nudibranch *Chromodoris reticulata*

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The structures and stereochemistry of six new diterpenes (**1**–**6**), two of which contain cyclic imine functionality, have been deduced by 2D NMR spectroscopy. The anatomical distribution of these, and of 17 other diterpenes (**7**–**23**) that were also isolated, has been investigated. The known compound aplysroeol-2 (**14**) was the major compound in the mantle tissue along with some dialdehydes, while the linear furan amblyofuran (**7**) was the only diterpene found solely in the internal organs. The presence of lactone-acetal-hemiacetal functionality in many of the isolated compounds is a consequence of the reactive dialdehydes present in the mollusc.

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Introduction

Nudibranchs from the order of Opisthobranchia (Mollusca: Gastropoda) are shell-less marine molluscs which lack physical defences against predation; and thus, may employ chemical defences to deter predators. They may be protected by metabolites obtained from dietary sources, commonly sponges, or from *de novo* biosynthesis.[1–4] For example, a predator–prey interaction has been determined for *Glossodoris atromarginata* and its sponge diet that involves sesterterpene metabolites.[5] Additionally, isotope-feeding studies have revealed the capability of dorid nudibranchs such as *Dendrodoris limbata* to produce terpenoids from mevalonic acid via *de novo* biosynthesis.[6–8]

Numerous diterpenes have been reported from chromodorid nudibranchs,[1,2,9] and some of these metabolites show pronounced biological activity.[7,8,10–13] Molinski and Faulkner isolated the aromatic norditerpenes macfarlandins A and B,[14] closely related to aplysulfurin from *Aplysilla sulfurea*,[15,16] and macfarlandins C–E, related to metabolites found in *Dendrilla* sp.,[17] from *Chromodoris macfarlandi*. Their findings strongly suggested that *C. macfarlandi* may prey on two different sponges.[18] The Golgi-modifying properties of macfarlandin

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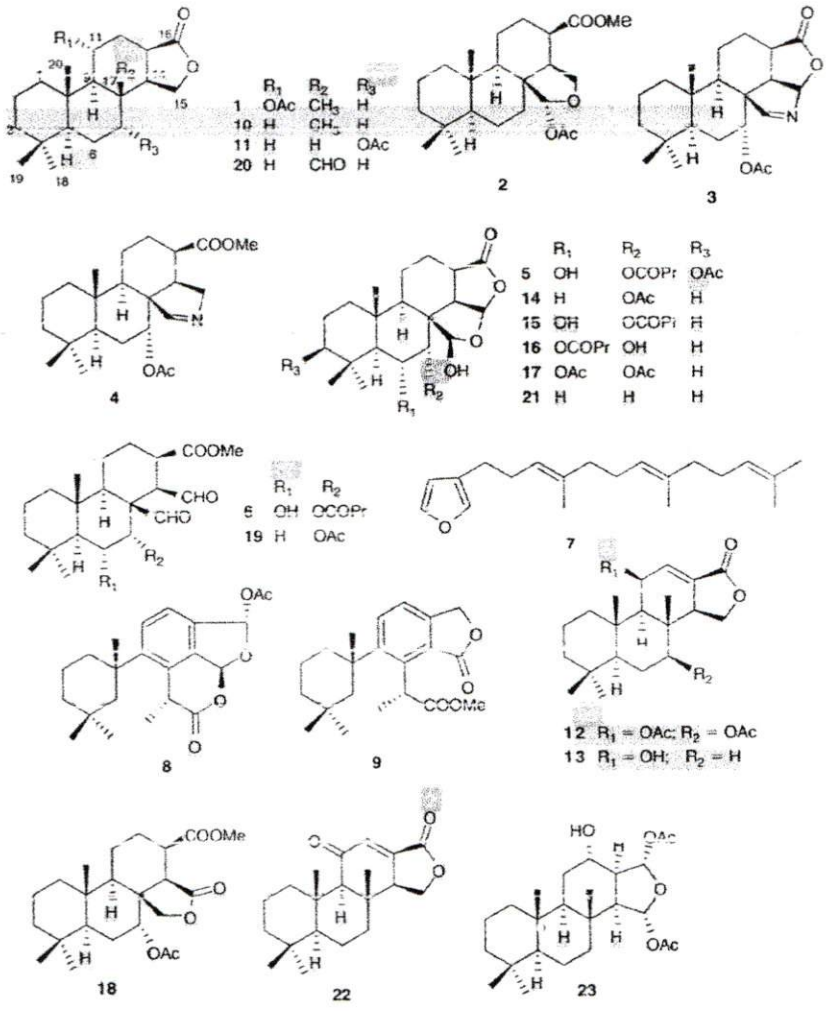

Fig. 1. Structures of diterpenoid metabolites isolated in the current study on the nudibranch *Chromodoris reticulata*.

E and of a synthetic analogue in which the hydroazuleno moiety is replaced by a *tert*-butyl group have been reported.[19] Cell biological studies of the dynamics of Golgi organization are providing fundamental insight into how these organelles play a key role in protein modification within the cell. An intriguing question to address is how this biological activity may relate to the ecological roles of macfarlandin E in both the mollusc and its dietary sponge.

We report six new diterpenes (**1**–**6**) together with 17 known diterpenes (**7**–**23**) (Fig. 1) isolated from two specimens of *Chromodoris reticulata*. The anatomical distribution of the diterpene compounds within the various tissue types of the mollusc, was explored by dissection and analysis of one of the two specimens. The data are compared with our study[20] on a specimen of a *Chromodoris* mollusc (species taxonomy possibly *reticulata*) which had earlier provided the two diterpenes **19** and **24**, along with the four known metabolites **10**, **14**, **18**, and **25** (Figs 1 and 2).

Results and Discussion

Two large specimens of *Chromodoris reticulata* were collected by SCUBA from the Gneerings Reef, offshore from Mooloolaba, in South East Queensland. One nudibranch was extracted with acetone to investigate the total chemistry and gave a terpene-rich organic extract that was fractionated by silica flash chromatography (hexanes/EtOAc), followed by normal phase HPLC (NP-HPLC) using hexanes/EtOAc. Using these separation protocols, three new (**1**–**3**) and 15 known metabolites were obtained. The known metabolites were identified by comparison of NMR data with the literature; these were ambliofuran (**7**),[21–23] aplysulfurin (**8**),[15,16] membranolide (**9**),[24]

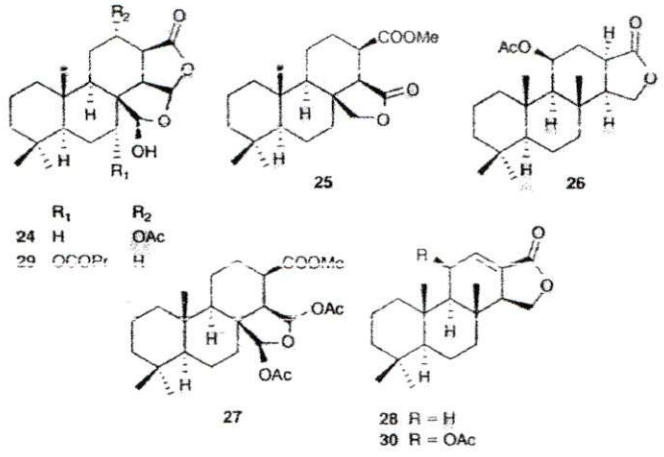

Fig. 2. Structures of diterpenoid metabolites isolated in earlier sponge or nudibranch studies, or from synthetic transformations.

spongian-16-one (**10**),[25] 7α-acetoxy-spongian-16-one (**11**),[22] dorisenone D (**12**),[13] 11β-hydroxyspongi-12-en-16-one (**13**),[26] aplyroseols-2, 3 and 5 (**14**–**16**),[13,22,27] dendrillol-2 (**17**),[23] aplyroseol-9 (=7α-acetoxydendrillol-3) (**18**),[28] the aldehydes **19**[20] and **20**,[29] and dendrillol-1 (**21**).[23] Diterpenes **10**, **14**, and **18** were isolated in our earlier study on an unidentified chromodorid nudibranch.[20] The second nudibranch was dissected to separate the mantle tissue from the internal organs in order to probe the distribution of individual metabolites. Extraction of the mantle tissue using the same procedure as for the first sample resulted in the isolation of three new diterpenes (**4**–**6**), while two additional compounds, namely spongi-12-en-11,16-dione (**22**)[26] and 12-deacetyl-aplysillin (**23**),[30] were identified by comparison with literature data. All compounds isolated from the first specimen were also isolated from the second specimen.

Diterpene **1** displayed a sodiated molecular ion peak in the HR-ESI MS at *m/z* 385.2345, corresponding to the molecular formula $C_{22}H_{34}O_4$. Inspection of the NMR spectra (Tables 1 and 2) confirmed the presence of four methyl singlets (δ_H 0.93, 0.91, 0.86, 0.82), and an acetate methyl (δ_H 2.01), while two oxymethylene protons (δ_H 4.31) were linked by HSQC to a carbon at δ_C 67.6, and showed HMBC correlations to a carbonyl at δ_C 180.8. These data suggested a spongian-16-one substituted with an acetate group, but when compared with 7α-acetoxy-spongian-16-one (**11**),[22] the shift of the oxymethine proton (δ_H 5.17 for H11 in **1** compared with δ_H 4.92 for H7 of **11**[22]) supported a different substitution pattern. The 11-OAc was deduced by HMBC correlations from H9 and H12α to C11, and confirmed by 1D TOCSY and COSY experiments. A boat conformation was inferred for ring C, since H13 at δ_H 2.90 showed a large coupling (*J* 11.5 Hz) to H12β and so was axially-orientated. Additionally, H14 and H13 were eclipsed, also showing an 11.5 Hz coupling; while an NOE between H12β and Me-17 supported the proposed conformation.[20,29,31] In an alternative 'flattened' chair conformation, there would not be a large coupling between the equatorial H13 and either H12 proton, nor would an NOE between H12β and Me-17 be expected. Although such data must be used with care, an NOE between H9 and H14, but no equivalent NOE between H11 and H13, suggested that the 11-OAc was α-oriented. NOEs between H11/Me-20 and between H11/H1β agreed with this interpretation. The β orientation of H11 was consistent with a *J* of 5.0 Hz between H11 and H9; a dihedral angle of 127.3° was suggested by molecular modelling (*Chem Bio 3D Ultra* 12.0 (Cambridge)) using a MM2 force field for energy minimization to an RMS of 0.100). The data for **1** differed with the partial data reported for the synthetic **26** (with a β-OAc), prepared by hydrogenation of a diterpene isolated from *Spongia officinalis*.[26] Although we named **1** as 11α-acetoxyspongian-16-one in view of its relationship to the known **10**, we note that the 'spongianone' nomenclature prevalent in the literature for such lactones is unsatisfactory.

Diterpene **2** was obtained by NP-HPLC (hexanes/EtOAc, 80/20) and had the molecular formula of $C_{25}H_{36}O_5$ inferred from HR-ESI MS (*m/z* 415.2463 $[M+Na]^+$). The ^1H NMR spectrum showed the presence of three methyl singlets (δ_H 0.85, 0.83, 0.78), an acetate methyl (δ_H 2.11), a carboxymethyl (δ_H 3.65), an acetal proton (δ_H 6.44), and two oxymethylene protons (δ_H 3.95, 3.73). The data were similar to those of diterpene **27**, except for the oxymethylene proton signals instead of signals for a second acetal moiety.[29] HMBC correlations from the acetal proton (δ_H 6.44) to C7 (δ_C 37.4) and the OAc (δ_C 170.8) confirmed that the acetate group was attached to C17. The relative configuration of **2** was then explored.[20,31] A boat conformation was inferred for ring C since the axial H13 at δ_H 2.79 again showed a large coupling (*J* 12.3 Hz) to H12β. A 5.7 Hz coupling between H13 and H14 matched with a dihedral angle of approximately 60°,[29] while the NOE between H9 and H13 agreed with the measured inter proton distance of 2.2 Å. Inspection of molecular models revealed that H17 would show an NOE to Me-20 irrespective of the C17 configuration. However, there was a strong NOE from H17 to the H15 proton (δ_H 3.73) assigned as β owing to the small coupling (1.7 Hz) with H14 that results from a dihedral angle close to 90°. The β orientation of H17 was further confirmed by an NOE to H11β, and by the shifts of H7α and H7β (δ_H 1.36 and 2.35) that matched equivalent values in diterpene **27** (δ_H 1.39 and 2.54). This latter metabolite was previously isolated from the nudibranch *Ceratosoma brevicaudatum*,[29] now considered a species of the genus *Chromodoris*.[2]

Table 1. ^{1}H NMR data for diterpenes 1–6[A]

C	1 δ_H, m (J in Hz)[B]	2 δ_H, m (J in Hz)[B]	3 δ_H, m (J in Hz)[B]	4 δ_H, m (J in Hz)[C]	5 δ_H, m (J in Hz)[B]	6 δ_H, m (J in Hz)[D]
1	α 0.90 m β 1.43 m	α 0.86 m β 1.64 m	α 0.95 td (13.9, 3.8) β 1.77 m	α 0.95 m β 1.67 m	α 1.15 m β 1.76 m	α 0.97 m β 1.67 dt (13.3, 3.3)
2	α 1.59 m β 1.38 m	α 1.60 m β 1.42 m	α 1.65 m β 1.52 m	α 1.63 m β 1.48 m	α 1.73 m β 1.65 m	α 1.56 m β 1.47 m
3	α 1.13 td (13.5, 4.0) β 1.38 m	α 1.14 td (13.3, 3.6) β 1.38 m	α 1.21 td (13.3, 4.0) β 1.47 m	α 1.21 td (13.5, 3.5) β 1.44 m	4.50 dd (11.8, 4.8)	α 1.22 td (13.7, 3.9) β 1.38 dt (13.7, 3.3)
5	0.89 brd (11.5)	0.93 dd (12.3, 2.3)	1.42 dd (11.9, 2.0)	1.39 dd (13.5, 2.5)	1.60 d (11.3)	1.47 d (11.7)
6	α 1.60 m β 1.41 m	α 1.56 m β 1.15 m	α 1.96 ddd (14.7, 3.1, 2.0) β 1.56 m	α 1.99 dt (15.0, 3.5) β 1.68 m	4.26 ddd (11.3, 6.0, 2.6)	4.09 ddd (11.7, 4.3, 3.0)
7	α 1.03 td (12.0, 4.0) β 1.70 dt (12.0, 3.5)	α 1.36 m β 1.35 m	4.61 t (3.1)	4.75 t (2.5)	4.95 d (2.6)	6.07 d (3.0)
9	1.17 d (5.0)	1.40 m	1.45 dd (11.9, 2.3)	1.64 m	1.46 dd (13.5, 2.9)	1.46 dd (12.3, 2.5)
11	5.17 td (5.0, 2.0)	α 1.63 m β 1.43 m	α 0.77 m β 1.66 m	α 0.98 m β 1.60 m	α 1.45 m β 1.98 qd (13.5, 4.4)	1.72 m; 1.76 m
12	α 2.17 ddd (16.0, 8.5, 2.0) β 1.98 ddd (16.0, 11.5, 5.0)	1.83 m	α 1.74 m β 2.21 m	1.80 m	α 1.58 m β 2.39 ddd (13.9, 4.4, 2.4)	α 1.61 m β 2.47 dq (13.6, 2.0)
13	2.90 td (11.5, 8.5)	2.79 dt (12.3, 5.7)	2.72 ddd (12.0, 9.1, 2.6)	2.74 m	2.76 m	3.29 td (5.6, 2.0)
14	2.31 m	2.24 m	2.92 dd (12.0, 6.6)	2.47 m	2.76 m	2.56 d (5.6)
15	4.31 m	α 3.95 dd (9.7, 6.9) β 3.73 dd (9.7, 1.7)	6.30 dd (6.6, 1.3)	α 4.00 ddd (17.0, 9.0, 2.0) β 3.71 ddd (17.0, 6.0, 2.5	6.06 m	9.73 s
17	0.91 s, 3H	6.44 s	7.99 d (1.3)	7.57 br s	5.53 d (1.8)	10.00 s
18	0.86 s, 3H	0.85 s, 3H	0.81 s, 3H	0.80 s, 3H	1.10 s, 3H	1.08 s, 3H
19	0.82 s, 3H	0.78 s, 3H	0.83 s, 3H	0.83 s, 3H	1.05 s, 3H	0.95 s, 3H
20	0.93 s, 3H	0.83 s, 3H	1.03 s, 3H	0.89 s, 3H	1.01 s, 3H	0.85 s, 3H
OCOCH_3	2.01 s, 3H	2.11 s, 3H	2.15 s, 3H	2.11 s, 3H	2.07 s, 3H	
COOCH_3		3.65 s, 3H		3.67 s, 3H		3.67 s, 3H
OCOCH_2CH$_2$CH$_3$					2.45 m	2.38 m
OCOCH$_2$$CH_2CH_3$					1.73 m	1.68 m
OCOCH$_2$CH$_2$$CH_3$					1.01 t (7.4), 3H	0.98 t (7.4), 3H
6-OH					1.44 d (6.0)	1.56[E]
17-OH					2.87 d (1.8)	

[A]Chemical shifts [ppm] referenced to $CHCl_3$ (δ_H 7.26).
[B]Data recorded at 500 MHz.
[C]Data recorded at 750 MHz.
[D]Data recorded at 900 MHz.
[E]Detected in DQFCOSY by correlation to H6.

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