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Terpenoid metabolites of the Australian nudibranch G*oniobranchus coi* (Risbec, 1956)

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Dedicated to the late Professor Yoel Kashman

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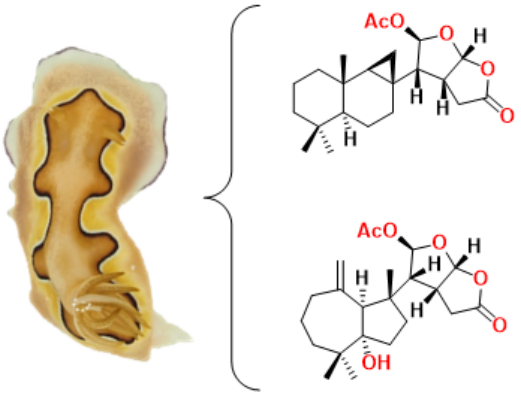
Abstract

We describe the isolation and structural elucidation of two new diterpenes from an organic extract of the nudibranch *Goniobranchus coi*. Plausible routes to the biosynthesis of cyclopropyl-functionalized marine terpenes are presented.



Keywords: Nudibranch, G*oniobranchus coi*, marine sponges, marine-derived terpenes, diterpenes, nuclear magnetic resonance spectroscopy, biosynthesis, marine natural-product isolation.

Introduction

Diterpenes and norditerpenes are bioactive natural products isolated from marine sponges and which can be sequestered by predatory shell-less mollusks. Many of these terpenoids are typically associated with the sponge orders *Dictyoceratida* and *Dendroceratida* and with nudibranchs of the family *Chromodoridae.*[1] Biosynthetically, the protonation of geranylgeranyl diphosphate initiates a concerted cyclization sequence, ultimately generating the parent spongian-diterpene skeleton,[2] which may then undergo extensive rearrangements leading to the trimethylcyclohexane, perhydroazulene or perhydronapthalene scaffolds frequently associated with marine-derived terpenes. In this article, we describe the chemistry of two specimens of *Goniobranchus coi*, a mollusc which is associated with Queensland marine locations, and report the isolation of two new terpenes.

Recently, we reported the isolation of 5,9-epoxydendrillolide A (**1**) and 10-oxonordendrillolide A (**2**) from *G. coi*, with both of these terpenes exhibiting conformational averaging in their ^{1}H NMR spectra at room temperature. For (**1**), low temperature NMR experiments together with DFT calculations supported interconversions of a twisted-chair conformation with two different chair conformers.[3] A follow-up article reported a second epoxide 10,20-epoxydendrillolide A (**3**) together with an aldehyde **4** identified as the ring-opened product of the C-10 epimer of **3**. The relative configuration of epoxide **3** was explored by comparison with those of *m*-chloroperbenzoic acid oxidation products derived from dendrillolide A (**5**),[4] while aldehyde (**4**) yielded the norketone (**2**) on storage.[5] The current research extends the chemical description of *G. coi* with structural and stereochemical investigation of two additional terpenes, 5-hydroxydendrillolide A (**6**) and the cyclopropyl-containing dendrillolide F (**7**). Representative marine natural products that contain a cyclopropyl group within the terpene framework include polyrhaphin C (**8**),[6] its 12-acetoxy derivative (**9**),[7] dendrillolide E (**10**),[8] cheloviolin **(11**),[9] omriolide B (**12**),[10] and verrielactone (**13**).[11] The structures of **1**-**13** are shown in **Figure 1**.

Results and Discussion

Two specimens of *G. coi* were collected from Percy Isles (2015) and Coolum (2017), Australia. Specimens were dissected into their mantle and viscera; each body part was extracted with acetone and the extract concentrated under vacuum, with the residues then partitioned between distilled water and EtOAc. The two mantle extracts were combined based on similar ^{1}H NMR spectra, as were the viscera extracts. Subsequent NP-HPLC purification provided a total of 19 oxygenated rearranged terpenes, of which 13 were identified as known terpenoids (see **Supplementary Material** for structures of known compounds), while the 4 new compounds **1**-**4** were reported in our earlier publications.[3,5]

The diterpene 5-hydroxydendrillolide A (**6**) (**Figure 1**) was isolated from mantle tissue as a colorless oil and displayed an adduct ion at *m/z* 415.2086 $[M+Na]^+$ from HRESIMS, which afforded the molecular formula $C_{22}H_{32}O_6$. When these data were compared to the molecular formula of dendrillolide A (**5**), the additional oxygen suggested the presence of either an ether or a hydroxy group.

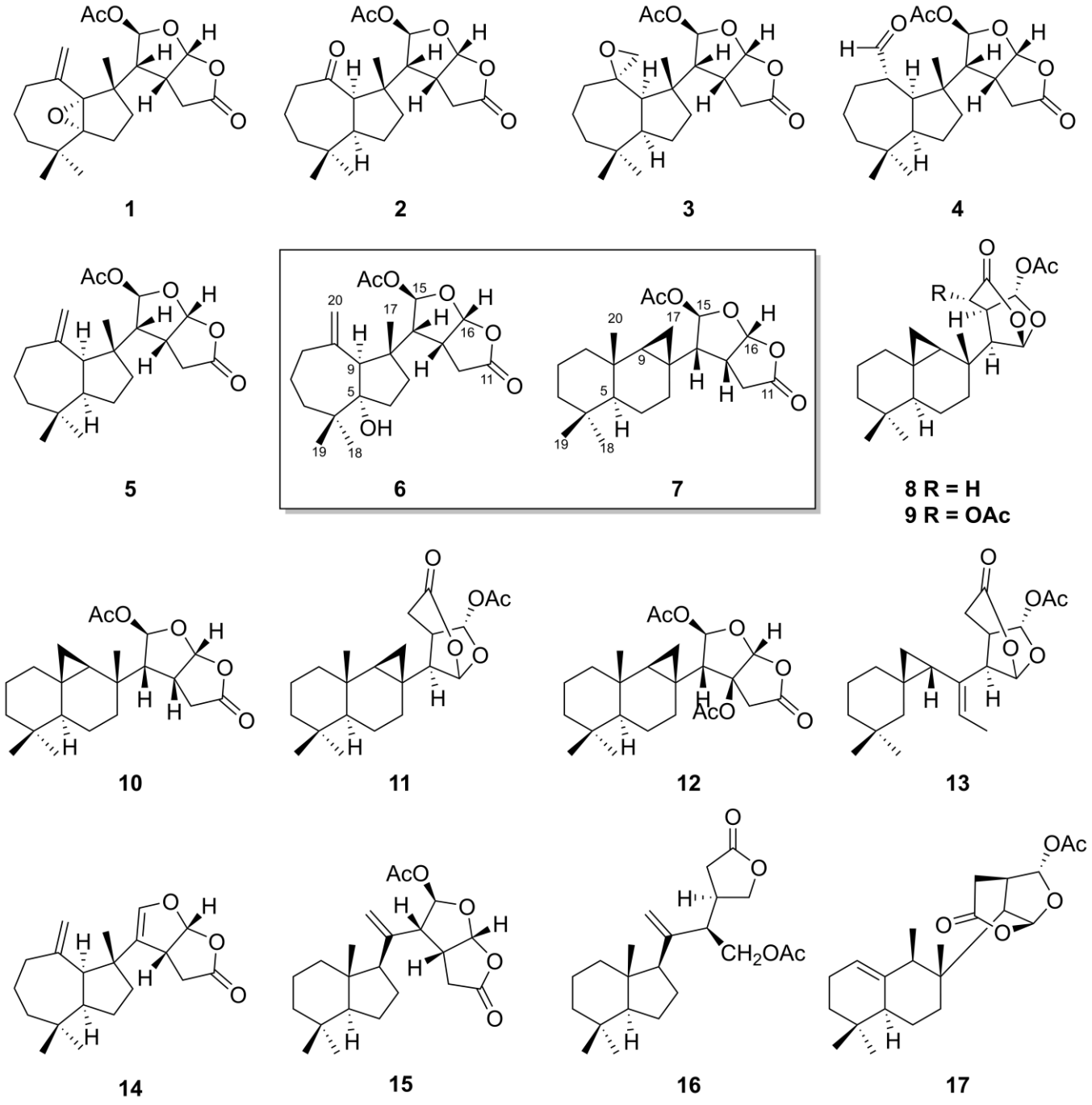


Figure 1. Structures of marine terpenes; structures **1-7** and **14-17** are of metabolites isolated from *G. coi* while structures **8-13** are rearranged terpenes reported in the literature that contain cyclopropyl functionality.

Inspection of the ^{1}H NMR spectrum (**Table 1**) revealed an acetoxy methyl singlet at δ_H 2.10, two acetal proton signals at δ_H 6.58 (d, *J* = 6.5 Hz) and 6.05 (d, *J* = 4.1 Hz), as well as exomethylene signals at δ_H 4.64 (d, *J* = 2.3 Hz) and 4.86 (d, *J* = 2.3 Hz). Comparison with the ^{1}H NMR spectrum of dendrillolide A (**5**) supported a dendrillane-derived carbon skeleton, however, signals for the bridgehead methine of the perhydroazulene motif seen in **5** (δ_H 1.76, δ_C 54.6) were missing. Instead, there was a tertiary hydroxy group at C-5, based on HMBC correlations from the three methyl signals at δ_H 0.99 (Me-18), 0.94 (Me-19) and 0.98 (Me-17) to a carbon signal at δ_C 87.6. NOESY correlations observed between H-13/H-14, H-13/H-16 and H-14/Me-17 confirmed the configuration of the 2,8-dioxabicyclo[3.3.0]octane moiety (**Figure 2**). NOESY correlations were

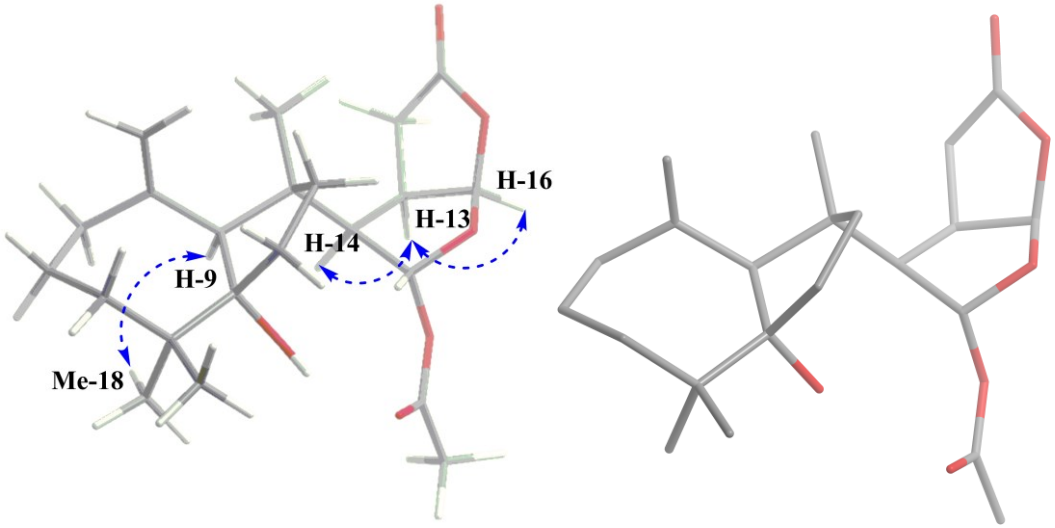


Figure 2. Molecular modelling for 5-hydroxydendrillolide A (**6**), showing key NOESY correlations and with hydrogen atoms omitted from the model for clarity.

also observed between H-9/Me-18 and so confirmed the assignment of Me-18. The configuration of the tertiary hydroxy group was not explored in detail owing to the small quantity (0.4 mg) of the sample. From a biosynthetic perspective, **1** and **6** may represent related P450 oxidation products of dendrillolide A (**5**); consequently, it is plausible that C-5 has an *S*-configuration as shown in **Figure 1**.

Dendrillolide F (**7**) was isolated as a colorless oil from both mantle and viscera extracts, and displayed a sodiated molecular ion peak $[M+Na]^+$ in the HRESIMS at *m/z* 399.2154 which corresponded to a molecular formula of $C_{22}H_{32}O_5$. The ^{1}H NMR spectrum presented an acetate methyl singlet at δ_H 2.07 and cyclopropyl signals at δ_H 0.28, 0.47 and 0.76, each of the latter integrating for one proton as shown in **Table 1**. Comparison of the ^{1}H NMR spectroscopic data of **7** with literature examples of rearranged diterpenes, in particular polyrhaphin C (**8**) and omriolide B (**12**), which possess a cyclopropyl functionality, aided in establishing the structure.[6-11] HMBC correlations from the H-5 bridgehead methine (δ_H 0.72) to the signals for C-18 (δ_C 33.1), C-19 (δ_C 21.5) and C-20 (δ_C 21.1) were consistent with a perhydronaphthalene motif. HMBC correlations from the cyclopropyl methylene protons (H_2-17) to C-7 (δC 29.8), C-9 (δ_C 35.6), C-10 (δ_C 32.2) and C-14 (δ_C 54.8) confirmed the cyclopropyl group to be fused across the C-8/C-9 bond, as seen in cheloviolin (**11**)[9] and omriolide B (**12**).[10] The heterocyclic component was next elucidated, in particular gCOSY correlations linked H-14 (δ_H 1.99) to H-13 (δ_H 3.29) and H-15 (δ_H 6.20), as well as H-13 to H-16 (δ_H 6.11) and H_2-12 (δ_H 2.99 and 2.67), suggesting a 2,8-dioxabicyclo[3.3.0]octane moiety, similarly seen in dendrillolide A (**5**)[4] and dendrillolide E (**10**).[8] A 2,7-dioxabicyclo[3.2.1]octane moiety as seen in polyrhaphin C (**8**)[6] and cheloviolin (**11**)[9] was also considered, but was rejected since the ^{13}C value observed for the lactone carbonyl (δ_C 174.7) did not fit within the typical range observed for a δ-lactone carbonyl (δ_C 165-168); instead, the value supported a γ-lactone. The 2,8-dioxabicyclo [3.3.0]octane ring system was further confirmed through HMBC correlations from H-15 to C-13 (δ_C 39.9) and the acetate carbonyl at δ_C 169.2, as well as correlations from H-16 to C-12 (δ_C 29.6), C-14 (δ_C 54.8), C-15 (δ_C 100.8), and the lactone carbonyl at δ_C 174.7 (C-11). The 2,8-dioxabicyclo[3.3.0]octane moiety was shown to be linked to the perhydronaphthalene motif at C-8/C-14, though HMBC correlations from H-14 (δ_H 1.99) to C-9 (δ_C 35.6) and C-17 (δ_C 11.9), as well as H-15 (δ_H 6.20) to C-8 (δ_C 19.0).

NOESY correlations between H-14/H-16, H-13/H-16 and H-13/H-14 revealed that these protons were all on the same face, establishing the configuration of the 2,8-dioxabicyclo[3.3.0]octane ring system. The

configuration of the cyclopropyl group was determined through NOESY correlations between H-14/H-17b and H-17a/Me-20, placing the cyclopropyl group on the same face as Me-20.

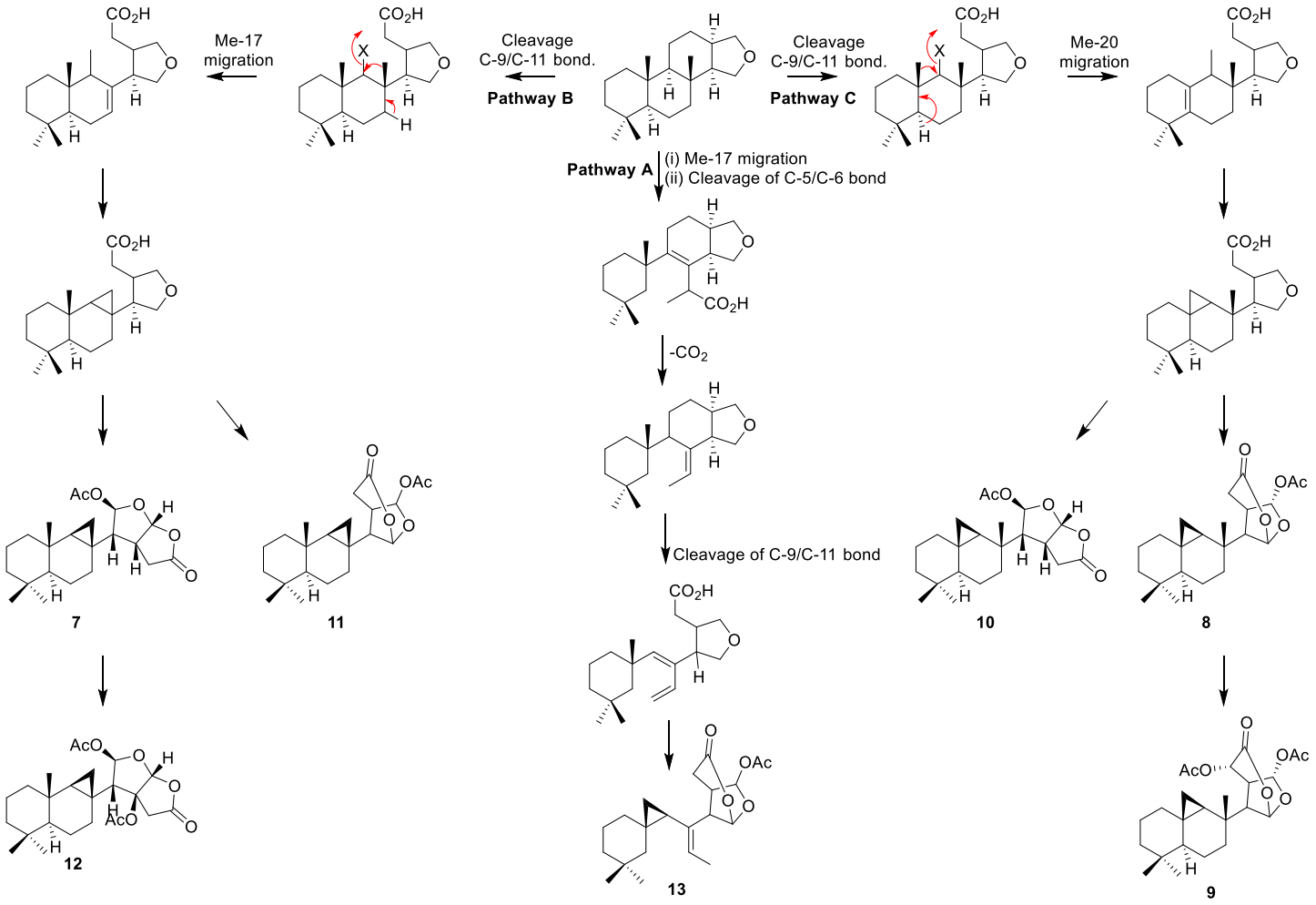
Table 1. ^{1}H and ^{13}C NMR Assignments of 5-Hydroxydendrillolide A (**6**) and Dendrillolide F (**7**) [a,b]

	6		**7**	
Position	**^{13}C**	**^{1}H (mult., *J,* Hz)**	**^{13}C**	**^{1}H (mult., *J,* Hz)**
1	37.1, CH_2	a2.37, m	43.4, CH_2	a1.60, m
		b1.84, m		b1.41, m
2	27.9, CH_2	a1.72, m	20.0, CH_2	a1.68, m
		b1.47, m		b1.50, m
3	39.4, CH_2	a1.64, m	41.7, CH_2	a1.41, m
		b1.41, m		b1.22, m
4	40.3, C	-	33.1, C	-
5	87.6, C	-	54.4, CH	0.72, m
6	34.2, CH_2	a2.06, m	17.1, CH_2	a1.44, m
		b1.60, m		b0.82, m
7	34.7, CH_2	a2.20, m	29.8, CH_2	a1.77, m
		b1.38, m		b1.57, m
8	46.8, C	-	19.0, C	-
9	64.4, CH	2.76, m	35.6, CH	0.76, dd (5.8, 9.9)
10	150.9, C	-	32.2, C	-
11	175.3, C	-	174.7, C	-
12	29.0, CH_2	a2.72, dd (17.9, 10.1)	29.6, CH_2	a2.99, dd (4.9, 18.7)
		b2.50, dd (17.9, 9.3)		b2.67, dd (10.4, 18.7)
13	42.0, CH	3.12, m	39.9, CH	3.29, m
14	54.4, CH	2.73, m	54.8, CH	1.99, dd (3.3, 8.9)
15	97.7, CH	6.58, d (6.5)	100.8, CH	6.20, d (3.3)
16	104.5, CH	6.05, d (4.1)	106.9, CH	6.11, d (6.0)
17	24.4, CH_3	0.98, s	11.9, CH_2	a0.47, dd (5.3, 5.8)
				b0.28, ddd (0.8, 5.3, 9.9)
18	20.7, CH_3	0.99, s	33.1, CH_3	0.83, s
19	28.9, CH_3	0.94, s	21.5, CH_3	0.73, s
20	115.5, CH_2	4.86, d (2.3)	21.1, CH_3	0.93, s
		4.64, d (2.3)		
15-O*CO*CH_3	169.5, C	-	169.2, C	-
15-OCO*CH_3*	21.1, CH_3	2.10, s	21.1, CH_3	2.07, s

[a]Chemical shifts (ppm) referenced to $CHCl_3$ at δ_H 7.26, δ_C 77.16. [b]At 700 MHz.

In the literature of marine natural products, cyclopropyl-functionalized diterpenes are proposed to originate from the spongian scaffold[2] and can be formed through one of three pathways (**Scheme 1**). Pathway A begins with migration of Me-17 and oxidative cleavage of the C-5/C-6 bond. Subsequently, the loss of CO_2

leads to the gracilin A carbon skeleton, while oxidative cleavage of the C-9/C-11 bond provides the spongionellin skeleton, from which verrielactone (**13**) can be derived.[11] Pathway B starts with an oxidative cleavage of the C-9/C-11 bond and Me-17 migration, from which the C-8/C-9 cyclopropyl can be formed. We also propose an alternative pathway in which, following oxidative C-9/C-11 bond cleavage, the loss of HX from C-9 (where X = OH, OR, etc., leaving group), and subsequent cyclopropyl formation from Me-17, a final cyclization gives cheloviolin (**11**) and the new metabolite **7**. Pathway C follows similar steps to pathway B, with oxidative cleavage of the C-9/C-11 bond, however, instead, migration of Me-20 occurs to provide the C-9/C-10 cyclopropyl structure, where subsequent cyclization provides **8** – **10**.



Scheme 1. Putative biosyntheses of cyclopropyl-containing marine terpenes.

With the characterization of **6** and **7** complete, we revisited the original ^{1}H NMR spectra of the mantle and viscera tissues in order to assess the anatomical distribution of the various metabolites. Nudibranch species may have a broad sponge diet and so contain diverse sponge metabolites in their gut tissues, yet selectively accumulate individual metabolites in their mantle tissue as defensive compounds. Localization of individual metabolites in mantle or mantle-rim tissue may, therefore, be an indicator of a potential ecological role for the metabolites.[12] In the current study, the majority of terpene metabolites were found in both mantle and viscera tissues. The new metabolite 5-hydroxydendrillolide A (**6**) and the known compound dendrillolide C (**14**) were only present in mantle tissue while the viscera tissue contained the known

compounds norrisolide (**15**),[13] cheloviolene C (**16**),[9] macfarlandin D (**17**)[4] (Figure 1), and polyrhaphin C (**8**).[6] The small amounts of **6** and **7** that were isolated in this study precluded further investigation to probe the palatability or toxicity of these two metabolites.

Conclusions

The analysis of two specimens of *Goniobranchus coi*, collected from the East coast of Australia, has yielded the 5-hydroxy derivative of dendrillolide A from mantle tissue as well as a perhydronaphthalene analogue containing cyclopropyl functionality and which was isolated from both mantle and viscera. The biosyntheses of these terpenes, including a proposed alternative pathway, have been shown and discussed.

Experimental Section

General. Nudibranch specimens were collected using SCUBA at depths between ~2-16 m. All specimens were stored in individual specimen jars at -20 °C until dissection and extraction. Specific rotations were measured at 23 °C on a Jasco P-2000 polarimeter for solutions in $CHCl_3$ using a 1-millilitre cell (10-centimetre path length). NMR spectroscopic data were recorded on a Bruker Avance 500 spectrometer using a 5-millimetre SEI probe or a Bruker Avance DRX 700 MHz spectrometer with a 5-millimetre TXI Zgrad probe for solutions in $CDCl_3$ at 298K. Heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) data were acquired using a $^1J_{C\text{-}H}$ of 145 Hz, while HMBC spectra were acquired using $^nJ_{C\text{-}H}$ of 8 Hz. Positive and negative ion electrospray mass spectra were determined using either a Bruker Esquire HCT 3D ion trap instrument for low-resolution electrospray ionization mass spectrometry (LRESIMS) or a MicrOTOF-Q or an Orbitrap Elite instrument for high-resolution electrospray ionization mass spectrometry (HRESIMS) with MeOH as solvent. Normal–phase high-performance liquid chromatography (NP-HPLC) was undertaken using a Waters 515 pump connected to a Gilson 132 series refractive index detector with a Phenomenex Luna (5 µm, 10 × 250 mm) column, using isocratic elution conditions at flow rates between 1-2 mL/ min. Silica gel 60 G and silica TLC plates F_{254} were purchased from Merck. Solvents were either distilled or were HPLC grade.

Two frozen nudibranch specimens (collection numbers: Percy Isles #1426 and Coolum #1647) were each dissected into viscera and mantle body segments. Individually, each body segment was finely chopped, extracted with acetone (3 x 2 mL), and sonicated (5 min). The extracts were then filtered through cotton wool, reduced to an aqueous suspension before partitioning between H_2O (2 mL) and EtOAc (4 x 2 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered through cotton wool and concentrated under N_2 to yield a yellow oil (29.3 mg and 24.1 mg) from the mantle tissues and an orange oil (51.1 mg and 43.4 mg) from the viscera tissues. The ^{1}H NMR profile of each extract was recorded. The extracts of the mantle were then combined, and the viscera extracts likewise combined. Each extract was subjected to NP-flash column chromatography with a gradient elution of 100% hexanes to 100% MeOH *via* CH_2Cl_2 and EtOAc. Selected fractions were combined and further subject to NP-HPLC to yield the purified compounds. For the mantle, fractions eluting from hexanes/CH_2Cl_2 (1:1) to CH_2Cl_2 (100%) were screened by ^{1}H NMR and were separated by NP-HPLC (25% EtOAc in hexanes) to provide 15-dendrillactol (6.55 mg), 15-epidendrillactol (6.55 mg), dendrillolide C (0.2 mg), dendrillolide A (21.1 mg), macfarlandin C (0.9 mg), macfarlandin E (0.4 mg), 12-desacetoxypolyrhaphin A (2 mg), aplyviolene (0.8 mg), 12-desacetoxyshahamin C (0.6 mg), 5,9-

epoxydendrillolide A (**1**: 0.8 mg), 5-hydroxydendrillolide A (**6**: 0.44 mg), dendrillolide F (**7**: 0.32 mg), aldehyde product (**4**: 0.9 mg), 10-oxonordendrillolide A (**2**: 1.2 mg), and 10,20-epoxydendrillolide A (**3**: 0.2 mg). For the viscera fractions eluting from hexanes/CH_2Cl_2 (1:1) to CH_2Cl_2/EtOAc (4:1) were separated by NP-HPLC (25% EtOAc in hexanes) yielding 15-dendrillactol (6.55 mg), 15-epidendrillactol (6.55 mg), dendrillolide A (**5**: 14.5 mg), macfarlandin E (0.6 mg), macfarlandin C (0.6 mg), 12-desacetoxypolyrhaphin A (1.2 mg), 12-desacetoxyshahamin C (0.5 mg), macfarlandin D (0.4 mg), aplyviolene (1.0 mg), polyrhaphin C (**8**: 0.2 mg), cheloviolene C (**16**: 0.1 mg), norrisolide (**15**: 0.2 mg), 5,9-epoxydendrillolide A (**1**: 0.6 mg), dendrillolide F (**7**: 0.1 mg), aldehyde product (**4**: 0.2 mg), 10-oxonordendrillolide A (**2**: 0.6 mg), and 10,20-epoxydendrillolide A (**3**: 0.2 mg).

*(+)-5-Hydroxydendrillolide A (**6**)*: colorless oil (0.44 mg); $[\alpha]^{21}_D$ + 19 (*c* 0.044, $CHCl_3$); ^{1}H NMR and ^{13}C NMR ($CDCl_3$, 700 MHz), (Results and Discussion, Table 1); HRESIMS *m/z* 415.2086 $[M + Na]^+$ (calcd. For $C_{22}H_{32}NaO_6$, 415.2091).

*(–)-Dendrillolide F (**7**)*: colorless oil (0.32 mg); $[\alpha]^{21}_D$ – 10 (*c* 0.032, $CHCl_3$); ^{1}H NMR and ^{13}C NMR ($CDCl_3$, 700 MHz), (Results and Discussion, Table 1); HRESIMS *m/z* 399.2154 $[M + Na]^+$ (calcd. For $C_{22}H_{32}NaO_5$, 399.2142).

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Supplementary Material

Copies of the 1D and 2D spectra of metabolites **6** and **7** are included in the Supplementary Material file associated with paper, together with a summary of terpene compounds isolated from *Goniobranchus coi.*

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