Chromolactol, an Oxygenated Diterpene from the Indo-Pacific Nudibranch *Goniobranchus coi*: Spectroscopic and Computational Studies*

Ariyanti S. Dewi, Gregory K. Pierens, Karen L. Cheney, Joanne T. Blanchfield, and Mary J. Garson

A School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Qld 4072, Australia.
B Research Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Marine Affairs and Fisheries, Jakarta 10260, Indonesia.
C Centre for Advanced Imaging, The University of Queensland, Brisbane, Qld 4072, Australia.
D School of Biological Sciences, The University of Queensland, Brisbane, Qld 4072, Australia.
E Corresponding author. Email: m.garson@uq.edu.au

A rearranged spongian diterpene chromolactol was obtained from the mantle extract of the Indo-Pacific nudibranch *Goniobranchus coi*. The structure of chromolactol, either 1a or 1b, which was investigated by extensive NMR experiments and by data comparison as well as by molecular modelling studies and density functional calculations, has a different relative configuration of the 2,8-dioxabicyclo[3.3.0]-octane ring compared with the co-metabolite norrisolide (2). A biosynthetic pathway leading to the preferred diastereomer of chromolactol (1a) is presented.

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**Introduction**

In many nudibranchs, the loss of a protective shell is compensated by the acquisition of chemical defences from food. Nudibranchs may elaborate the chemicals, thus making them more effective as toxins or as deterrents towards predators. Alternatively, nudibranchs may biosynthesise secondary metabolites (*de novo*), thereby providing a defence that is independent of diet.[1–2] The distastefulness and toxicity of nudibranchs are thought to be related to colour patterns used as warning signals (aposematism).[3–5]

In terms of chemistry, among the most intensively studied nudibranchs are those of the two closely related genera *Chromodoris* and *Goniobranchus*, which have been reported to contain furanososquiterpenes,[6,7] norditerpenes,[8–11] diterpenes,[9,11–27] sesterterpenes,[28,29] and occasionally macrolides.[30] Our group has investigated aplyroseol-type compounds from *Chromodoris* sp. and *G. reticulata* collected near Mooloolaba, south-east Queensland.[24,26] Further investigations on *G. reticulata* yielded chromoculatimines A and B, and a spongian-16-one derivative.[26] Other spongian diterpenes have been obtained from the extracts of *G. albopunctata* and *Doriprismatica atromarginata*.[11,23,27] Finally, chemical extracts from *G. verrieri* and *G. splendidus* yielded a diverse array of norditerpenes and diterpenes bearing gracilane skeletons.[9,10]

In the present paper, we report the chemical analysis of an extract from *G. coi* from Mackay, Queensland, and describe the structure elucidation of a new oxygenated diterpene chromolactol, which contains two distinct chiral domains. Our spectroscopic and computational investigations unambiguously determined the relative configuration within each chiral domain, but did not conclusively distinguish between candidate diastereomers 1a or 1b. However, biosynthetic expectations were in accordance with diastereomer 1a as the preferred structure.

**Results and Discussion**

The new diterpene chromolactol (Fig. 1) was isolated from the Et2O extract of a single specimen of *Goniobranchus coi* (sample code #1095) collected near Mackay, together with norrisolide (2),[12] cheloviolene C (3),[31] macfarlandin C (4),[32] and dendrillolide A (5).[33] The isolated metabolites were purified by normal-phase (silica) flash chromatography followed by normal-phase high-performance liquid chromatography (HPLC) separation. The structures of chromolactol and of the known metabolites were characterized by means of 2D NMR spectroscopic and mass spectrometric analysis and by comparison with literature data.

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Chromolactol was isolated as a colourless oil from normal-phase HPLC. The molecular formula of C_{20}H_{30}O_{4} (m/z 333.2075 [M – H]–) was identical to those of cheloviolenes A (6) and B (7). The 1H NMR of I revealed two acetol protons (δH 6.18 and 5.54), an exomethylene group (δH 4.92 and 4.88), three sp³ methine protons (δH 3.01, 2.76, 2.06), and three methyls at δH 0.67 (3H), 0.86 (6H). The heteronuclear multiple-bond correlation (HMBC) spectrum revealed alkene signals at δC 148.2 and 111.5, and a lactone carbonyl at δC 175.4. The exomethylene and the lactone contributed to two of the required six double-bond equivalents; therefore, the compound was tetracyclic.

The 1H and 13C NMR data (Table 1) for the A/B ring system of chromolactol matched closely those of norrisolide (2) [12] or cheloviolenec C (3); thus, a perhydroindane system was inferred. This conclusion was confirmed by correlation spectroscopy (COSY) and HMBC data (Fig. 2). The relative configurations at C-5, C-8, and C-9 in chromolactol were deduced to be the same as those of 2 and 3 based on the close similarity of the NMR data. Furthermore, there were nuclear Overhauser effect spectroscopy (NOESY) correlations observed between H-12/H-13/H-16, and H-14/H-15, while the absence of W-coupling between H-13 and H-15 further supported the [3.3.0]-octane ring system. HMBC correlations from both H-14 and H-15 to C-13 (δC 45.2), from H-17a and H-17b to C-14 (δC 58.5) and to C-8 (δC 58.8) linked the exomethylene moiety to the perhydroindane system and to the 2,8-dioxabicyclo[3.3.0]-octane ring (Fig. 2).

The stereochemistry of the 2,8-dioxabicyclo[3.3.0]-octane ring system was next considered, comparing the relative configuration with those of norrisolide (2), macfarlandin C (4), and dendrillolide A (5), as well as those of cheloviolenes A (6) and B (7).

### Table 1. 1H and 13C NMR data (CDCl3) of chromolactol

<table>
<thead>
<tr>
<th>Position</th>
<th>δH/AC</th>
<th>δC/AC</th>
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<tbody>
<tr>
<td>1</td>
<td>1.67</td>
<td>m</td>
</tr>
<tr>
<td>2</td>
<td>1.10</td>
<td>m</td>
</tr>
<tr>
<td>3</td>
<td>1.59</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>1.52</td>
<td>m</td>
</tr>
<tr>
<td>5</td>
<td>1.45,</td>
<td>1.07</td>
</tr>
<tr>
<td>6</td>
<td>1.07</td>
<td>m</td>
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<tr>
<td>7</td>
<td>1.72,</td>
<td>1.04</td>
</tr>
<tr>
<td>8</td>
<td>1.65</td>
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<tr>
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<td>m</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>m</td>
</tr>
<tr>
<td>11</td>
<td>–</td>
<td>m</td>
</tr>
<tr>
<td>12</td>
<td>2.86,</td>
<td>dd(18.6, 11.4)</td>
</tr>
<tr>
<td>13</td>
<td>2.75,</td>
<td>dd(18.6, 5.3)</td>
</tr>
<tr>
<td>14</td>
<td>3.01,</td>
<td>dd(11.4, 6.2, 5.3)</td>
</tr>
<tr>
<td>15</td>
<td>2.76,</td>
<td>br s</td>
</tr>
<tr>
<td>16</td>
<td>5.54</td>
<td>s</td>
</tr>
<tr>
<td>17</td>
<td>6.18,</td>
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<tr>
<td>18</td>
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<td>s</td>
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<td>19</td>
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<tr>
<td>22</td>
<td>0.67</td>
<td>s</td>
</tr>
<tr>
<td>15-OH</td>
<td>nd</td>
<td>–</td>
</tr>
</tbody>
</table>

A At 700 MHz, referenced to 1H in CDCl3 at δH 7.27 and δC 77.16.
B Coupling constant in Hertz in parentheses.
C Values taken from 2D NMR data.
D Not detected.

1m, multiplet; dd, doublet of doublets; s, singlet; br, broad; d, doublet.
domain rather than a perhydroindane moity, are rare examples of rearranged spongian diterpenes with a 2,8-dioxabicyclo-
[3.3.0]octane ring system that possess a trans configuration
between H-13 and H-14. In the original isolation work, the
relative configuration of cheloviolene A (6) was established by
an X-ray study that revealed that H-14 was trans to both H-13
and H-15.\textsuperscript{[31]} Confirmation of this relative configuration,
together with determination of the absolute configuration of
cheloviolene A, has recently been achieved by the Overman
group via an 11-step enantioselective total synthesis.\textsuperscript{[35]} Chelo-
viole B, which was initially reported as the C-15 epimer of 6,\textsuperscript{[31]} has also been the target of synthetic work by the Overman
group, which led to a revision of relative configuration to that
shown in 6.\textsuperscript{[31]} Cheloviolenes thus have the same relative
configuration in their 2,8-dioxabicyclo-[3.3.0]-octane rings,
but differ only in the configuration of this bicyclic moiety
relative to the perhydroazulene domain. The structure shown
for 7 was first presented in the literature as that of a sponge
diterpenoid chelonaplys B by Bohzin and Faulkner,\textsuperscript{[36]} however,
the published \textsuperscript{1}H NMR spectra of their metabolite were
shown to be identical to those of cheloviolene A.\textsuperscript{[31]}

In chromolactol, the \(J_{\text{13-14-H-16}}\) of 6.2 Hz was identical to that
in norrisolide (2) and cheloviolenes A (6) and B (7), and together
with the NOESY correlation between the two protons indicated
that H-13 and H-16 were cis-configured. The H-14 signal
appeared as a broad singlet (\(J_{\text{13-14-H-16}} < 1\) Hz), which matched
closely the equivalent signals of 6 and 7 (\(J\) values of 2.2
and 2.6 Hz respectively)\textsuperscript{[31]} and thereby the relationship between
H-13 and H-14 in 1 was established as trans. If H-13 and H-14 had
been cis-configured, a \(J_{\text{13-14-H-16}}\) of 6–10 Hz would have been
anticipated (cf. \textsuperscript{1}H NMR values of 9.4, 6.9, and 6.6 Hz for 2, 4,
and 5 respectively). Considering the C-15 configuration, the \(J_{\text{13-14-H-15}}\)
value of <1 Hz in 1 matched closely the reported values for
cheloviolenes A (6) and B (7).\textsuperscript{[31]} The small values of coupling
constants between H-13/H-14 and H-14/H-15 likely result from
the overall conformation of the 2,8-dioxabicyclo-[3.3.0]-octane
ring with a hydrogen bond between the hydroxy group and
the lactone. This hydrogen bonding causes each of the torsional
angles of H-14-C-14-C-13–H-13 and H-14-C-14-C-15–H-15 to be close to 90°.\textsuperscript{[36]}

Two candidate diastereomers of chromolactol (1a and 1b)
that differ in the stereochemistry of the 2,8-dioxabicyclo-
[3.3.0]-octane ring system were considered. Diastereomer 1a
possesses the same configuration as cheloviolene B, whereas
diastereomer 1b possesses the same configuration as chelovio-
lene A.

A Monte Carlo conformational search for each diastereomer
1a and 1b was undertaken with Merek Molecular Force Field
(MMFF) using \textit{Macromodel}\textsuperscript{[37]} and selected conformers
\textless 5 kcal mol\textsuperscript{-1} (1 cal = 4.184 J) of the global minimum: 1a,
20 conformers and 1b, 21 conformers) were optimized by
density functional theory (DFT) calculations at the B3LYP/6–
31+G(d,p) level with chloroform solvent (integral equation
formalism-polarisable continuum model, IEF-PCM) using
\textit{Gaussian} software.\textsuperscript{[38]} A single-point energy of the optimized
conformers was calculated using M062X/6–31+G(d,p) with
chloroform solvent (IEF-PCM)\textsuperscript{[39]} and was used in the weighted
chemical shifts. Four conformers for both 1a and 1b (M062X/6–
31+G(d,p) energy <3 kcal mol\textsuperscript{-1}) were selected to calculate the
chemical shifts using mpw1pw91/6–31+G(2d,p) with chloro-
form solvent (IEF-PCM). The Boltzmann-averaged \textsuperscript{1}H and \textsuperscript{13}C
chemical shifts were calculated by converting the magnetic field
tensors using linear scaling (\(\text{H}\) slope: –1.0717, intercept:
31.8721 and \(\text{C}\) slope: –1.0417, intercept: 186.3455). Next,
the mean absolute error (MAE) for both 1a and 1b was evaluated
using the calculated chemical shifts, revealing that there was
very little difference between the two diastereomers (\(\text{H}: \text{MAE}\)
of 0.08 ppm for both 1a and 1b isomers, and \(\text{C}: \text{MAE}\) of 1.8
and 1.9 ppm for 1a and 1b respectively). When the Boltzmann-
averaged \(\text{H}\) and \(\text{C}\) chemical shifts were examined using
DP4\textsuperscript{[40]} using both \(\text{H}\) and \(\text{C}\) chemical shifts, the probability
percentages were 1a 64.3 % and 1b 35.7 %; the preference for 1a
was slightly improved if the \(\text{C}\) chemical shifts alone were
analysed (68 %: 32 % for 1a: 1b).

Although these computational data clearly indicated that
chromolactol has the same partial relative configuration as the
2,8-dioxabicyclo-[3.3.0]-octane ring system of cheloviolenes
A or B, it was also apparent that the NMR data alone could not
distinguish between the two diastereomers 1a and 1b. The
two lowest-energy conformers of 1a (together representing
>98.6 % of the conformational population (ratio: 54 : 46); see
Supplementary Material) differed in rotation about the C-10–
C-14 bond, and hence in the orientation of the dioxabicyclooc-
tane ring system relative to the hydrocarbon fragment. Like-
wise for 1b, the two most stable conformers (representing
>98.1 % of the conformational population (ratio: 56 : 44)) also
differed in rotation about the C-10–C-14 bond. Consequently,
NOESY data could not be used to distinguish which of the two
candidate diastereomers corresponded to chromolactol. This
diterpene metabolite of \textit{G. coi} thus represents a rare example in
which computational results do not converge to a preferred
stereostructure. Jiao and coworkers recently determined the
partial relative configuration of two distinct chiral domains in
the cyclooxygenase-2 (COX-2)-inhibitory meroterpenoid
dysiarenone, but were unable to verify the overall relative con-
figuration.\textsuperscript{[41]} In our own work on the nudibranch \textit{Phyllidieila
pustulosa}, the antimarial diterpene pustulosaisonitrile-1
posed significant challenges for structure elucidation owing to
the C-6/C-7 stereochemical relationship and the presence of
two chiral domains separated by a flexible alkane linker. The
stereochemical problem was resolved by catalyst-controlled
stereoselective synthesis of two key diastereomers to establish
the relative configuration of the two independent chiral
domains.\textsuperscript{[42]}

The stereostructure initially deduced for cheloviolene A (6)
has been verified by total synthesis; in the same work, the
structure of cheloviolene B, which was originally assigned as
the C-15 epimer of 6, was revised by the Overman group. We
compared the \(\text{H}\) NMR data of chromolactol against those of
cheloviolenes A and B. Although the chemical shift for H-15 in
chromolactol (\(\delta_{\text{H}}\) 5.54) matched better the equivalent signal in
cheloviolene A (\(\delta_{\text{H}}\) 5.52) than in cheloviolene B (\(\delta_{\text{H}}\) 5.64), the
chemical shift for H-13 in chromolactol (\(\delta_{\text{H}}\) 3.01) matched
to the C-6/C-7 stereochemical relationship and the presence of
two chiral domains separated by a flexible alkane linker. The
stereochemical problem was resolved by catalyst-controlled
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chemical shift for H-13 in chromolactol (\(\delta_{\text{H}}\) 3.01) matched
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two chiral domains separated by a flexible alkane linker. The
stereochemical problem was resolved by catalyst-controlled
stereoselective synthesis of two key diastereomers to establish
the relative configuration of the two independent chiral
domains.\textsuperscript{[42]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig_2.png}
\caption{Selected COSY, HMBC, and NOESY correlations of chromolactol.}
\end{figure}
As the Overman synthetic study provided a rigorous assignment of NMR data for the cheloviolenes, we elected to test the experimental $^1$H and $^{13}$C NMR data for cheloviolene A against the theoretical data for this structure using the DP4 probability approach. It was found that the calculated $^1$H NMR data corresponding to the stereostructure $6$ matched closely with the experimental $^1$H NMR data of cheloviolene A (83.1 %), but in contrast, the calculated $^{13}$C NMR data corresponding to stereostructure $6$ were instead in better agreement with the experimental $^{13}$C NMR data of cheloviolene B (55.7 %) rather than with the data for cheloviolene A (44.3 %). Taking both $^1$H and $^{13}$C NMR data into account, the probability value was 79.6 % that the calculated data for stereostructure $6$ matched the experimental data for cheloviolene A. In other words, these results demonstrate that the DP4 NMR chemical shift calculations do not convincingly distinguish between diastereomers containing the same relative configuration in the 2,8-dioxabi-

cyclo-[3.3.0]-octane moiety but differing in the configuration of this domain relative to the chiral hydrocarbon fragment. The computational approach is generally considered a reliable indicator of (stereo)structure if the overall probability value is $>90 \%$.[43]

For chromolactol, diastereomer $1a$ may be preferred on biosynthetic grounds because it has the same configuration at C-13 and C-16 as many other oxygenated diterpenes (cf. 3). As shown in Scheme 1, the biosynthesis of $1a$ may proceed by opening of ring C of a spongiane precursor $8$, followed by contraction of ring B to yield the norrisane skeleton $9$.[12,44]

Oxidation of the tetrahydrofuran ring to a lactol followed by ring opening to an aldehyde allows epimerization at C-14. In this way, the cis configuration of H-13 and H-14 that is usually associated with oxygenated diterpenes can be changed to trans. Closure of ring D produces $1a$ with the overall relative configuration shown. Alternatively, epimerization at C-13 followed by ring closure establishes the configuration shown in $1b$.

Scheme 1. Proposed biosynthetic pathway of $1a$ and $1b$ showing overall relative configurational outcomes.
Conclusions
We have reported a new rearranged spongiom diterpene, chromolactol from the mantle extract of *Goniobranchus coi*. The relative configuration of the [3,3,0]-dioxabicyclooctane ring in chromolactol is identical to that observed in cheloviolenes A or B. Investigation of the relative configuration of chromolactol via NMR data analysis yielded two plausible diastereomers (1a, 1b). Neither the calculated MAE values nor the probability values defined by DP4 were able to differentiate conclusively between the two diastereomers. However, the overall relative configuration shown in diastereomer 1a is preferred to that of diastereomer 1b on biosynthetic grounds.

Experimental

**General Experimental Procedure**
Specific rotations were measured at 23°C on a Jasco P-2000 polarimeter for solutions in CHCl₃ using a 1-mL cell (10-cm path length). NMR data were measured on Bruker Avance 500 and 700-MHz spectrometers (5-mm inverse probe) for solutions in CDCl₃ at 298 K. Heteronuclear single quantum correlation (HSQC) and HMBC data were acquired using a J_C–H of 145 Hz, whereas HMBC spectra were acquired using J_C–H of 8 Hz. Positive- and negative-ion electrospray mass spectra were determined using either a Bruker Esquire HCT instrument for low-resolution electrospray ionization mass spectrometry (LRESIMS) or a MicrOTOF-Q instrument for high-resolution electrospray ionization mass spectrometry (HRESIMS) with MeOH as solvent. Normal-phase HPLC (NP-HPLC) was undertaken using a Waters 515 pump connected to a Gilson 132 series refractive index detector with a Waters 8413 small-chromatography column, and using isocratic elution conditions at flow rates of 1 mL/min. Silica gel 60 G and silica TLC plates (0.04 CHCl₃). 1H NMR (CDCl₃, 100 MHz) and 13C NMR (CDCl₃, 250 MHz) data were measured on Bruker Avance 500 spectrometers. Low-resolution electrospray ionization mass spectrometry (HRESIMS) with m/z 333.2071 [M – H]⁻ was obtained for the mantle tissue of *G. coi*.

**Biological Material**

*Goniobranchus coi* (#1095) (0.68 g) was collected from the Mackay region, Queensland, in October 2014, frozen, and stored at −20°C until extraction. The specimen was dissected into mantle and gut before extraction.

**Extraction and Purification**

The mantle tissue of *G. coi* (Mackay #1095; 0.49 g) was extracted in acetone (7 × 3 mL). The extract was reduced to an aqueous suspension, extracted with Et₂O (3 × 3 mL), dried over anhydrous Na₂SO₄ and concentrated under N₂ to give crude extract. The mantle extract (5.3 mg) was subjected to NP-HPLC (20% EtOAc/hexanes) and yielded dendrillolide A (0.6 mg), macfarlandin C (1.3 mg), norrisiolide (1.3 mg), chelovioliene C (0.2 mg), and chromolactol 1a (0.2 mg). Using similar methods, the gut extract (5.2 mg) afforded norrisiolide (2.0 mg), macfarlandin C (1.8 mg), chelovioliene C (1.3 mg), and dendrillolide A (0.1 mg).

**Chromolactol (1a)**

Colourless oil; [α]D⁰p −3 (c 0.04 CHCl₃). 1H NMR (CDCl₃, 700 MHz) and 13C NMR (CDCl₃, 700 MHz) data are presented in Table 1. m/z (HRESIMS) 333.2071 [M – H]⁻; calcd for C₂₀H₂₉O₄ 333.2075.

**Supplementary Material**
An image of *Goniobranchus coi*, copies of 1D and 2D NMR spectra of chromolactol (1) in CDCl₃, and details of computational studies are available on the Journal’s website.

**Conflicts of Interest**
The authors declare no conflicts of interest.

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**References**


