Oxygenated Sesquiterpenes From the Indo-Pacific Nudibranch Ardeadoris rubroannulata: Structure Revision of Pu’ulenal

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Abstract
Seven oxygenated sesquiterpenes 1 to 7 each with a drimane framework were isolated from an organic extract of the nudibranch Ardeadoris rubroannulata collected from Eastern Australia. The structure of pu’ulenal 2 was revised by 1D NOESY, providing a 9Z configuration, while isopu’ulenal 3 has the 9E configuration previously ascribed to pu’ulenal.

Keywords
Ardeadoris rubroannulata, sesquiterpene, terpenoid, NMR, x-ray crystallography, DFT, absolute configuration

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Introduction
The drimane sesquiterpene polygodial (1) is a distinctive and bioactive natural product isolated from plants¹-⁴ and from marine molluscs.⁵,⁶ In addition to anti-inflammatory activity, this hot, peppery tasting bicyclic dialdehyde displays antifeedant activity linked to adduct formation with biological amines.⁷-⁹ Although nudibranch molluscs may acquire drimane sesquiterpenes from dietary marine sponges, some species of nudibranchs have been shown to produce polygodial via de novo biosynthesis.⁶,¹⁰-¹² Herein, we report a study on the chemistry of a single specimen of Ardeadoris rubroannulata, a brightly-coloured nudibranch collected from Hancock Reef, S.E. Queensland, Australia, the mantle of which was found to contain polygodial. We also report six other oxygenated terpenes, including pu’ulenal (2) for which a structure revision was undertaken following the isolation of the double bond isomer isopu’ulenal (3). To our knowledge, this work constitutes the first chemical investigation of A. rubroannulata.

Results and Discussion
An organic extract from a single specimen of A. rubroannulata (#1649) collected near Coolum, S.E. Queensland, Australia, provided pu’ulenal 2¹¹ and isopu’ulenal 3 (Figure 1) along with the known metabolites polygodial 1,¹⁶,¹⁴ drimenin 4,¹⁰,¹⁶ isodrimenin ⁵,¹⁷,¹⁸ cinnamolide ⁶,¹⁶ and euryfuran ⁷,¹⁹. The isolated metabolites were separated by normal phase HPLC (20-30% EtOAc in hexanes) following silica flash chromatography. Known compounds were identified through comparison of mass spectrometric data, literature and in-house NMR data. The structures of pu’ulenal 2 and isopu’ulenal 3 were defined following detailed spectroscopic analysis and led to a revision of the C-9/C-11 configuration for 2.

The oxygenated sesquiterpene pu’ulenal (2) was isolated as an amorphous white solid and displayed a sodiated peak in the HRESIMS at m/z 299.1611 [M + Na]⁺. The corresponding molecular formula of C₁₇H₂₄O₃, as well as an acetoxyl methyl singlet (11-OCOCH₃, δ 2.09) in the ¹H NMR spectrum suggested there was an acetate group. Three singlet methyl signals at δH 0.88 (Me-13), δH 0.93 (Me-14) and δH 0.98 (Me-15) confirmed the presence of the trimethyl substituted cyclohexane moiety. The 500 MHz HSQC and ¹H NMR spectra of 2 revealed a singlet aldehyde proton (H-12, δH 9.62) and signals for two alkenes at δH 6.76, δC 143.9 (H-7) and δH 7.13, δC 129.6 (H-11). In addition, the HMBC spectrum of 2 revealed two quaternary alkenes carbons (C-8 δC 136.0 and C-9 δC 127.9), indicating two separate alkenes in the structure

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There were also HMBC correlations from H-5 to C-3 and C-7, H-6 to C-7 and C-8, H-7 to C-9, H-11 to C-8, C-9, and the acetate carbonyl, while the aldehyde proton H-12 gave HMBC correlations to C-7, C-8, and C-9 (Figure 2).

The marine natural products literature contains a single report of the isolation of puleuvalen from the nudibranch Chromodoris albonotata by Schulte and Scheuer. Their study reported that the C-9/C-11 alkene of puleuvalen possessed an E-geometry based on observations following the addition of an NMR shift reagent (Eu(fod))3. However, when we conducted a 1D NOESY experiment in which H-11 was irradiated, NOE correlations to H-1a, H-1b and Me-15 were revealed, suggesting that puleuvalen instead possessed a Z-geometry (Figure 2). This was further supported through a 1D NOESY correlation observed to the acetoxy methyl (11-OCOCH3) on irradiation of the aldehyde proton H-12.

Fortuitously, crystallization of a sample of 2 was achieved from 10% EtOAc/hexanes and a suitable crystal then subjected to x-ray crystallographic analysis at the Australian Synchrotron. Processing of this data produced the crystal structure shown in Figure 3, confirming that the C-9/C-11 alkene of puleuvalen possesses a Z-geometry, and thereby correcting the initial erroneous assignment of the configuration by Schulte and Scheuer. To confirm the C-9/C-11 alkene E-geometry of 3, 1D NOESY experiments were performed, irradiating H-7, H-11 and H-12. Unexpectedly the only NOE correlation observed from these experiments was between H-7 and H-12, while the anticipated correlation between H-11 and H-12 was not observed (Figure 2). This suggested that the s-trans conformer of the C-7/C-8/C-12 α,β-unsaturated aldehyde in 3 is favoured over its s-cis conformer, a phenomenon that has been previously observed in polygodial (1), which possesses a similar structural motif.

The conformation of 3 was explored using molecular modelling and DFT calculations. This study suggested that isopuleuvalen (3) possesses two major conformers (Figure 4), in one of which the aldehyde adopts the s-trans form (~99%), while the other conformer adopts the s-cis orientation (~1%). The preference for the s-trans conformer could arise due to a number of different effects such as secondary orbital interactions of the complex conjugated system present in 2 and 3 or through intramolecular attractions between H-11 and the carbonyl oxygen of C-12. This significant preference for the s-trans form also explains the large 1H chemical shift difference between the H-11 protons of 3 (δH 8.28) and 2 (δH 7.13). In 3, H-11 is directly in the plane of the C-12 carbonyl group and would be subject to the effects of its deshielding cone.
Fortuitously both drimenin 4 and isodrimenin 5 crystallized after HPLC isolation, and their absolute configurations were therefore determined by x-ray crystallographic analysis and found as expected to be (5S,10S) (Figures 5 & 6). Since the original structural studies on isodrimenin 4,17,18 reported 1H NMR data alone, we provide below the 13C NMR data to supplement the NMR assignment.

### Experimental

**General:** 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 a Bruker Avance 500 MHz or a Bruker Avance III HD700 MHz spectrometer (5 mm TCI inverse cryoprobe) for solutions in CDCl3 at 298 K. HSQC and HMBC data were acquired using a 1JCH of 145 Hz, while HMBC spectra were acquired using 3JCH of 8 Hz. Low resolution electrospray ionization mass spectrometry (LRESIMS) was performed on a Thermo LCQ Fleet ion trap spectrometer in positive mode. High resolution electrospray ionization mass spectrometry (HRESIMS) was performed on an Orbitrap Elite instrument with a standard ESI source (sodium formate) with MeOH as solvent. Normal phase high performance liquid chromatography (NPHPLC) was undertaken using a Waters 515 pump connected to a

| Table 1. 1H and 13C NMR Data of Terpenes 2 to 3. |
|-----------------|-----------------|-----------------|-----------------|
| Pu’ulenalin (2) | Isopu’ulenalin (3) |
| Position       | δ1Habc,d         | δ13Cd           | δ1Habc,d         | δ13Cd           |
| 1              | 1.81, br d (12.5) | 36.5, CH2       | 2.65, m          | 37.8, CH2       |
|                | 1.53, m          | -               | 1.65, m          | -               |
| 2              | 1.62, m          | 18.5, CH2       | 1.63, m          | 18.9, CH2       |
| 3              | 1.62, m          | -               | 1.54, m          | -               |
|                | 1.48, m          | 42.0, CH2       | 1.45, m          | 41.6, CH2       |
|                | 1.21, td (13.2, 4.1) | -       | 1.23, m          | -               |
| 4              | 1.48, m          | 33.4, C         | -               | 33.4, C         |
| 5              | 1.48, m          | 25.5, CH2       | 25.5, m          | 25.5, CH2       |
| 6              | 2.24, ddd (21.0, 11.5, 3.4) | -       | 2.30, ddd (20.3, 12.2, 2.7) | -               |
| 7              | 6.76, dd (4.9, 3.4) | 143.9, CH       | 6.79, dd (5.9, 154.0, 2.7) | CH               |
| 8              | 136.0, C         | -               | 136.2, C         | -               |
| 9              | 127.9, C         | -               | 125.4, C         | -               |
| 10             | 36.0, C          | -               | 39.0, C          | -               |
| 11             | 7.13, s          | 129.6, CH       | 8.28, s          | 133.9, CH       |
| 12             | 9.62, s          | 191.4, CH       | 9.47, s          | 193.4, CH       |
| 13             | 0.88, s          | 32.4, CH3       | 0.92, s          | 33.2, CH4       |
| 14             | 0.93, s          | 21.4, CH3       | 0.94, s          | 22.0, CH4       |
| 15             | 0.98, s          | 19.3, CH3       | 1.05, s          | 19.1, CH4       |
| 11- OAc        | 167.8, C         | -               | 167.1, C         | -               |
| 11- OAc        | 2.09, s          | 20.7, CH3       | 2.17, s          | 20.9, CH3       |

*Chemical shifts referenced to 1H at δH 7.27 and δC 77.16.
*Coupling constant in Hz.
*At 500 MHz.
*Values taken from 2D NMR data.
*At 700 MHz.

Figure 2. Selected HMBC and NOESY correlations observed for pu’ulenalin 2 and isopu’ulenalin 3.

Figure 3. ORTEP representation of the crystal structure of pu’ulenalin 2 shown with 50% probability ellipsoids.
Gilson 132 series refractive index detector with a Waters μPorasil (10 μm, 7.8 x 300 mm) or a Phenomenex Luna (5 μm, 10 x 250 mm) column, and using isocratic elution conditions at flow rates between 1 to 2 mL/min. Silica gel 60 G and silica TLC plates F254 were purchased from Merck. Solvents were either distilled or were of HPLC grade.

**Animal material:** The nudibranch specimen *A. rubroannulata* (#1649) was collected from Hancock Shoal, near Coolum, S.E. Qld in January 2017 and stored at −20 °C until extraction.

**Extraction and isolation of metabolites:** The frozen nudibranch (5.3 g) was dissected into its mantle (2.1 g), gut (1.3 g) and mantle rim (1.9 g). The individual body parts were finely chopped then extracted with acetone (6 x 3 mL) and sonicated (5 min). Each extract was filtered through cotton wool and concentrated to an aqueous suspension before partitioning between H2O (2 mL) and EtOAc (7 x 3 mL). The organic layer was dried over anhydrous Na2SO4, filtered through cotton wool and evaporated under N2 to yield a light orange oil for the mantle (49.6 mg), a dark green oil for the gut (15.7 mg) and a dark orange oil for the mantle rim (256.1 mg). NP flash column chromatography, with a stepwise solvent gradient (hexanes: hexanes/DCM: DCM: DCM/EtOAc: MeOH) was used to further separate the mantle extract. Fractions eluting from hexanes (100%) provided euryfuran 7 (0.8 mg). Fractions eluting from hexanes/DCM (1:1) and hexanes/DCM (1:4) were separated by NP HPLC (20% EtOAc in hexanes) to give drimenin 4 (1.2 mg), isodrimenin 5 (2.6 mg), cinnamolide 6 (0.6 mg), polygodial 1 (1.7 mg), pu’u’ulenal 2 (4.3 mg) and isopu’ulenal 3 (0.8 mg).

**Pu’ulenal (2)**
White crystals. [α]D: −48 (c 0.18 CHCl3). HR-ESI-MS m/z [M + Na]+ calcd. for C17H24NaO3: 299.1618; found: 299.1611. 1H NMR (CDCl3, 500 MHz) and 13C NMR (CDCl3, 500 MHz) are presented in Table 1.

**Isopu’ulenal (3)**
Colorless oil. [α]D: −45 (c 0.02 CHCl3). HR-ESI-MS m/z [M + Na]+ calcd. for C17H24NaO3: 299.1618; found: 299.1618. 1H NMR (CDCl3, 500 MHz) and 13C NMR (CDCl3, 700 MHz) are presented in Table 1.

**Crystal Structure Determination of Pu’ulenal 2, Drimenin 4 and Isodrimenin 5**
Data were collected at the MX1 beamline of the Australian Synchrotron with Silicon Double Crystal monochromated radiation at 100(2) K (λ = 0.7107 Å)20. Data integration and reduction were undertaken with XDS.21 Subsequent computations were carried out using Olex2.22 Structures were solved with ShelXT23 and refined and extended with ShelXL24. Carbon-bound hydrogen atoms were included in idealised positions and refined using a riding model. The Flack25 parameter unambiguously confirmed the absolute structures. Crystallographic data is summarized below, and the CIFs...
have been deposited at the Cambridge Crystallographic Data Centre with CCDC numbers 2107013 to 2107015 for 2, 4 and 5, respectively. These data are available free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1 EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk

Crystal data for C17H24O3 (M = 276.36 g/mol): orthorhombic, space group P212121 (no. 19), a = 6.2730(12) Å, b = 11.436(2) Å, c = 20.720(4) Å, V = 1486.4(5) Å³, Z = 4, T = 100.15 K, μ(MoKα) = 0.083 mm⁻¹, Δcalc = 1.235 g/cm³, 27 409 reflections measured (3.932° ≤ 2Θ ≤ 63.486°), 4489 unique (Rint = 0.0603, Rsigma = 0.0357) which were used in all calculations. The final R1 was 0.0473 (I > 2σ[I]) and wR2 was 0.1302 (all data).

Crystal data for C15H22O2 (M = 234.32 g/mol): orthorhombic, space group P212121 (no. 19), a = 7.0990(14) Å, b = 7.7390(16) Å, c = 23.307(5) Å, V = 1280.5(4) Å³, Z = 4, T = 100(2) K, μ(synchrotron) = 0.078 mm⁻¹, Δcalc = 1.212 g/cm³, 21 314 reflections measured (5.508° ≤ 2Θ ≤ 56.566°), 3106 unique (Rint = 0.0363, Rsigma = 0.0198) which were used in all calculations. The final R1 was 0.0411 (I > 2σ[I]) and wR2 was 0.1123 (all data).

Crystal data for C15H22O2 (M = 234.32 g/mol): orthorhombic, space group P212121 (no. 19), a = 7.0990(14) Å, b = 7.7390(16) Å, c = 23.307(5) Å, V = 1280.5(4) Å³, Z = 4, T = 100(2) K, μ(synchrotron) = 0.078 mm⁻¹, Δcalc = 1.216 g/cm³, 18 090 reflections measured (5.548° ≤ 2Θ ≤ 56.566°), 2919 unique (Rint = 0.0396, Rsigma = 0.0229) which were used in all calculations. The final R1 was 0.0342 (I > 2σ[I]) and wR2 was 0.0815 (all data).

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Author Contributions

PTN carried out the experimental investigation, including NMR studies, and was involved in conceptualization of the study; GKP was involved in the NMR studies and undertook the modelling study; JKC undertook the x-ray crystallographic study; MJG was involved in data analysis and conceptualization of the study; all authors contributed to preparation of the manuscript and approved the final version.

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This article does not contain any studies with human or living animal subjects.

Statement of Informed Consent

There are no human subjects in this article, and informed consent is not applicable’

Supporting Information

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Trial Registration

Not applicable, as no clinical trials were involved.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Barnes CS, Loder JW. The structure of polygodial: a new sesquiterpene dialdehyde from Polygonum hydropiper L. Aust J Chem 1962;15(2):322-327.
2. Kubo I, Lee YW, Pettei M, Pilkiewicz F, Nakanishi K. Potent army-worm antifeedants from the East African Warbugia plants. J Chem Soc Chem Commun. 1976;(24):1013-1014.
3. Kioy D, Gray AI, Waterman PG. A comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of Warbugia ugandensis and W. stuhlmannii. Phytochemistry. 1990;29(11):3535-3538.
4. Rihak KJ, Bissember AC, Smith JA. Polygodial: a viable natural product scaffold for the rapid synthesis of novel polycyclic pyrrole and pyrrolidine derivatives. Tetrahedron. 2018;74(12):1167-1174.
5. Cimino G, De Rosa S, De Stefano S, Sodano G. The chemical defense of four Mediterranean nudibranchs. Comp Biochem Physiol. 1982;73B(2):471-474.
6. Cimino G, De Rosa S, De Stefano S, Sodano G, Villani G. Dorid nudibranch elaborates its own chemical defense. Science. 1983;219(4589):1237-1238.
7. Asakawa Y, Dawson GW, Griffiths DC, et al. Zhong-Ning Z1 activity of drimane antifeedants and related compounds against...
aphids and comparative biological effects and chemical reactivity of (-) and (+)-polygodial. *J. Chem. Ecol.* 1988;14(10):1845-1855.

8. Cimino G, Spinella A, Sodano G. Identification of an intermediate in the reaction between polygodial and methylamine in biomimetic conditions. *Tet Lett.* 1984;25(37):4151-4152.

9. Caprioli V, Cimino G, Colle R, Gavagnin M, Sodano G, Spinella A. Insect antifeedant activity and hot taste for humans of selected natural and synthetic 1,4-dialdehydes. *J Nat Prod.* 1987;50(2):146-151.

10. Gavagnin M, Mollo E, Castelluccio F, Ghiselin MT, Calado G, Cimino G. Can molluscs biosynthesize typical sponge metabolites? The case of the nudibranch *Doriopsilla areolata*. *Tetrahedron.* 2001;57(46):8913-8916.

11. Fontana A, Tramice A, Cutignano A, Ippolito G, Gavagnin M, Cimino G. Terpene biosynthesis in the nudibranch *Doriopsilla areolata*. *J Org Chem.* 2003;68(6):2405-2409.

12. Gaspar H, Cutignano A, Ferreira T, Calado G, Cimino G, Fontana A. Biosynthetic evidence supporting the generation of terpene che- modiversity in marine molluscs of the genus *Doriopsilla*. *J Nat Prod.* 2008;71(12):2053-2056.

13. Schulte GR, Scheuer P. Defense allomones of some marine mollusks. *Tetrahedron.* 1982;38(13):1857-1863.

14. Rodriguez B, Zapata N, Medina P, Viñuela E. A complete $^1$H and $^{13}$C NMR data assignment for four drimane sesquiterpenoids isolated from *Drimys winterii*. *Magn. Res. Chem.* 2005;43(1):82-84.

15. Rukachaisirikul V, Khamthong N, Sukpondma Y, et al. Cyclohexene, diketopiperazine, lactone and phenol derivatives from the sea fan-derived fungi *Nigrospora* sp. PSU-F11 and PSU-F12. *Arch Pharmacal Res.* 2010;33(3):375-380.

16. Hollinshead DM, Howell SC, Ley SV, Mahon M, Ratcliffe NM, Worthington PA. The diels-alder route to drimane related sesquiterpenes: synthesis of cinnamolide, polygodial, isodrimeninol, drimenin and warbuganal. *J Chem Soc Perkin Trans I.* 1983:1579-1589.

17. White JD, Burton LP. Syntheses of the insect antifeedant (+)-cinnamodial and the drimane sesquiterpenoids (+)-isodrimenin and (+)-fragrolide. *J Org Chem.* 1985;50(3):357-364.

18. Akita H, Oishi T. Ozonolysis of phenolic dehydroabietane derivatives - syntheses of optically active (+)-confertifolin, (+)-valdiviolide, (+)-winterin, (+)-isodrimenin, and (+)-pallescensin A. *Chem Pharm Bull.* 1981;29(6):1580-1587.

19. Hochlowski JE, Walker RP, Ireland C, Faulkner DJ. Metabolites of four nudibranchs of the genus *Hypselodoris*. *J Org Chem.* 1982;47(1):88-91.

20. McPhillips TM, McPhillips SE, Chiu HJ, et al. Blu-Ice and the distributed control system: software for data acquisition and instrument control at macromolecular crystallography beamlines. *J Synchrotron Radiation.* 2002;9(6):401-406.

21. Kabsch W. Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J Appl Cryst.* 1993;26(6):795-800.

22. Dolomanov OV, Bourhis LJ, Gildea RJ, Howard JAK, Puschmann H. OLEX2: a complete structure solution refinement and analysis program. *J Appl Cryst.* 2009;42(2):339-341.

23. Sheldrick GM. *SHELXT*-Integrated space-group and crystal-structure determination. *Acta Cryst.* 2015;A71(1):3-8.

24. Sheldrick GM. Crystal structure refinement with *SHELXL*. *Acta Cryst.* 2015;C71(1):3-8.

25. Flack HD. On enantiomorph-polarity estimation. *Acta Cryst.* 1983;A39(6):876-881.