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New sesquiterpenoid isonitriles from three species of phyllidid nudibranchs

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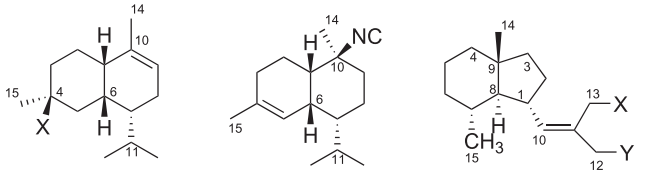
ABSTRACT

Chemical investigation of the two nudibranch species *Phyllidiella pustulosa* and *Phyllidia ocellata* collected in Queensland, Australia, provided new stereoisomers of 4-isocyano-9-amorphene (**1**) and of 10-isocyano-4-amorphene (**2**), respectively. A specimen of *Phyllidia picta* collected from Bali, Indonesia, contained the axane sesquiterpenoids pictaisonitrile-1 (**3**) and pictaisonitrile-2 (**4**). The planar structures were elucidated using 1D and 2D NMR spectroscopy, while relative configurations were established using NOESY correlations, coupling constant data, and comparison with literature data.

1. Introduction

Marine organisms, in particular molluscs, are a prolific source of structurally diverse natural products. Chemoecological studies on molluscs have investigated the sequestration of biologically potent chemicals from their sponge diets [1]. Nudibranchs of the genus *Phyllidia* and their dietary sponges have been found to contain terpene isonitriles and isothiocyanate metabolites [2,3] possessing pronounced bioactivities including antimalarial [4–6], antifouling [7] and other biological activities [1,8] Herein, we report the isolation of sesquiterpene isonitriles from species of phyllidiid nudibranchs collected either in Australia or in Indonesia. New stereoisomers of 4-isocyano-9-amorphene (**1**) and of 10-isocyano-4-amorphene (**2**) were identified from *Phyllidiella pustulosa* and from *Phyllidia ocellata*, while a specimen of *Phyllidia picta* collected from Bali, Indonesia contained the axane sesquiterpenoids pictaisonitrile-1 (**3**) and pictaisonitrile-2 (**4**).



1; X = NC
5; epimer at C-4
6; X = NCS

2
7; epimer at C-10
8; epimer at C-6

3; X = NC; Y = H
4; X = H; Y = NC
9; X = H; Y = SCN

2. Experimental

2.1. General experimental procedures

Analytical reagent grade solvents for extraction and flash column chromatography were distilled prior to use. Thin layer chromatography (TLC) was carried out on Silica gel 60 F_{254} coated aluminium-backed plates (Merck Art. 5554). The plates were visualised under UV (254 and 365 nm) and coated with vanillin stain reagent [vanillin (15 g), 96% ethanol (250 mL) and concentrated sulfuric acid (2.5 mL)] followed by heating with hot air. Normal phase flash chromatography was carried out on Merck silica gel 60 (0.063–0.200 mm, 70–230 mesh ASTM). Gradient elution used hexanes to methanol under pressure of compressed air with combinations of the resulting fractions guided by TLC. Normal phase (NP) high performance liquid chromatography (HPLC) was conducted with a Waters 515 pump in combination with a Gilson® 132 refractive index detector. All HPLC separations were performed using a semi preparative Waters μPorasil® column 10 μm (7.8 × 300 mm) or Phenomenex Luna 5 μ silica column (250 × 10 mm); with isocratic elution conditions using premixed, filtered and degassed mobile phases. Flow rates were 2 mL/min for mobile phases up to 20% EtOAc in hexanes. Low resolution electrospray ionisation mass spectrometry (LRESIMS) was performed on a Bruker Esquire HCT 3D ion trap spectrometer in positive or negative ion mode. High resolution electrospray ionisation mass spectroscopy (HRESIMS) was performed on a MicroTof-Q instrument with a standard ESI source (sodium formate). Proton nuclear magnetic resonance (^{1}H NMR) spectra were

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recorded on Bruker Avance spectrometers operating at 700 or 900 MHz using 5 mm SEI probes. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker Avance DRX 900 spectrometer with a 5 mm TXI Zgrad probe. NMR spectra were acquired in base-filtered deuterated chloroform ($CDCl_3$) solvents and referenced to solvent signals at δ_H 7.26 (^{1}H) and δ_C 77.16 (^{13}C). Chemical shifts (δ) were recorded in parts per million (ppm) and coupling constants (J values) were measured in Hertz (Hz). Two dimensional NMR (2D NMR) data were acquired from Bruker Avance 700 and 900 MHz instruments. Gradient enhanced HMBC (geHMBC) and HSQC (geHSQC) NMR were obtained with 8 to 64 transients per increment with the evolution delay set at $^nJ_{CH}$ of 4 Hz or 8 Hz (geHMBC) and $^1J_{CH}$ of 135 Hz (geHSQC). Gradient COSY (gCOSY) data were recorded with 8 to 32 transients per increment with a pulse delay of 2.0 s. NOESY NMR spectra were obtained with 32 to 64 transients per increment, a recycle time between scans of 3.4 s and mixing times of 0.6 or 1.5 s. Selective 1D-TOCSY experiments used mixing times of 0.04 s, 0.08 s or 0.12 s. Specific rotation $[\alpha]_D$ measurements were performed on a Jasco P-2000 polarimeter, with the Sodium D-line of 589.5 nm. Measurements were recorded using an optical path length of 10 cm at 22–25 °C for solutions in $CHCl_3$.

2.2. Extraction and isolation

2.2.1. Extraction and isolation of isocyanides 1 and 2 from P. pustulosa

Four individual *P. pustulosa* specimens (7 g total) were collected from Mooloolaba and chopped finely into pieces, extracted with acetone (5 × 10 mL) and sonicated (5 mins). The extract was filtered through a cotton wool plug and evaporated to an aqueous suspension before partitioning between Et_2O (3 × 8 mL) and H_2O (8 mL). The combined organic layer was dried with anhydrous Na_2SO_4, filtered through cotton wool and concentrated under N_2 to give an orange oil. The crude extract (240 mg) was subjected to NP flash chromatography and the fractions eluting from 100% hexane, hexane/DCM (4:1) and hexane/DCM (1:4) were further purified with NP HPLC (2% EtOAc) and gave both of the new isocyanides **1** and **2** in addition to the known sesquiterpenes axisonitrile-3 [9,10], axisothiocyanate-3 [9–11], 1-isocyanoaromadendrane [12], 1-isothiocyanatoaromadendrane [12], 1-isocyanatoaromadendrane [13,14], 9-isocyanopupukeanane [15], 2-isocyanoallopupukeanane [16], and 2-isocyanotrachyopsane [17].

(+)-(1*S,4*S**,6*S**,7*R**)-4-Isocyano-9-amorphene (1)**: Colorless oil (0.5 mg); $[\alpha]_D$ + 10 (*c* 0.18, $CHCl_3$); GC/MS *m/z* $[M]^+$231 (12), 216 (30), 204 (26), 189 (28), 161 (38), 107 (58), 81 (100); HRESIMS *m/z* 254.1879 $[M + Na]^+$ (calcd for $C_{16}H_{25}NNa$, 254.1879); ^{1}H NMR ($CDCl_3$, 700 MHz) and ^{13}C NMR ($CDCl_3$ 700 MHz) see Table 1.

(−)-(1*S,6*R**,7*R**,10*S**)-10-Isocyano-4-amorphene (2)**: Colorless oil (0.4 mg); $[\alpha]_D$ − 11 (*c* 0.01, $CHCl_3$); GC/MS *m/z* $[M]^+$ 231 (12), 216 (30), 204 (26), 189 (28), 161 (38), 107 (58), 81 (100); HRESIMS *m/z* 254.1892 $[M + Na]^+$ (calcd for $C_{16}H_{25}NNa$, 254.1879); ^{1}H NMR ($CDCl_3$, 700 MHz) and ^{13}C NMR ($CDCl_3$ 700 MHz) see Table 1.

2.2.2. Extraction and isolation of sesquiterpene isocyanide 2 from P. ocellata

Fourteen specimens of *P. ocellata* (35 g) were chopped finely into pieces, extracted with acetone (5 × 10 mL) and sonicated (5 mins). The extract was filtered through cotton wool plug and evaporated to an aqueous suspension before partitioning between Et_2O (3 × 8 mL) and H_2O (8 mL). The combined organic layer was dried with anhydrous Na_2SO_4, filtered through cotton wool and concentrated under N_2 to give an orange oil (248 mg). The crude extract was subjected to flash chromatography on silica gel with stepwise gradient elution from 100% hexanes to 100% MeOH, and monitored by GC/MS and ^{1}H NMR. The fractions eluting from 100% hexanes and hexanes/DCM (4:1) were further purified through NP HPLC (2% EtOAc) yielding isocyanide **2**, together with nine known sesquiterpenes axisonitrile-3 [9,10], axisothiocyanate-3 [9-11], halichonadin C [18], acanthene B [19], axisonitrile-2 [20], 2-isocyanoclovene [21], 4,5-epi-10-isocyanoisodauc-6-ene [21], epipolasin A [22], and 7-isocyano-7,8-dihydro-α-bisabolene [13].

2.2.3. Extraction and isolation of pictaisonitriles from P. picta

A single specimen of *P. picta* (30 mm) from Bali was finely chopped, extracted with acetone (5 × 10 mL), and sonicated (2 min). The extracts were filtered through cotton wool and reduced to an aqueous suspension before partitioning between H_2O (10 mL) and Et_2O (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered through cotton wool, and evaporated under N_2 to yield a brown gum (13 mg). The extract was further separated by NP flash column chromatography and the fraction eluting from hexanes:DCM (3:1) was further purified by NP-HPLC (1% EtOAc in hexanes) to afford an inseparable mixture (~2.9:1) of pictaisonitrile-1 (**3**) and pictaisonitrile-2 (**4**) (0.2 mg), together with 11-isocyano-7β*H*-eudesm-5-ene [20], the isonitrile analogue of epipolasin A [23] and 4-isocyanoeudesm-11-ene [24].

Pictaisonitriles-1 and -2 (**3,4**): colorless oil (0.2 mg); $[\alpha]_D$ + 12 (*c* 0.03, $CHCl_3$); HRESIMS *m/z* 254.1879 $[M + Na]^+$ (calcd for $C_{16}H_{25}NaN$, 254.1879); ^{1}H NMR ($CDCl_3$, 900 MHz) and ^{13}C NMR ($CDCl_3$ 900 MHz) see Table 1.

3. Results and discussion

3.1. Structure elucidation of a new 4-isocyano-9-amorphene diastereomer (1)

A new isocyanide **1**, isolated as a colorless oil by NP HPLC (2% EtOAc/ hexanes) had a molecular formula of $C_{16}H_{25}N$ confirmed by HRESIMS measurement of the ion peak at *m/z* 254.1879 $[M + Na]^+$. The ^{1}H NMR spectrum displayed a distinctive alkene signal at δ_H 5.43 (br s), a vinyl methyl at δ_H 1.63 (br s), a methyl adjacent to an isocyano group at δ_H 1.39 (br s) and two isopropyl methyls at δ_H 0.95 (d, J = 6.6 Hz) and 0.88 (d, J = 6.6 Hz). The HSQC and HMBC data identified 12 protonated carbon signals, including those for four methyl groups. There was a trisubstituted alkene (δ_C 132.9 (C) and 123.9 (CH)), a quaternary carbon at δ_C 58.4, as well as the signal for an isocyano carbon at δ_C 153.5. Two fragments were established from the HMBC correlations of Me-15 at δ_H 1.39 to C-3, C-4 and C-5 in ring A as well as Me-14 to C-1, C-9, C-10 in ring B (Fig. 1). An isopropyl group was also determined through the HMBC correlations from Me-12 and Me-13 to C-11 and C-7. Finally, the COSY data established the planar structure of **1** as shown in Fig. 1. A comparison of carbon chemical shifts with data for 4α-isocyano-9-amorphene (**5**) [16] supported the same amorphene carbon skeleton, although there were differences in the ^{13}C chemical shift values for Me-15 adjacent to the isocyano functionality (δ_C 30.5 in **1** vs. δ_C 24.4 in **5**) that supported a change in configuration at C-4.

The NOE correlation of H-7 to the bridgehead proton at H-1 revealed that H-7 was axial, and therefore that the isopropyl group was in an equatorial orientation. Then, Me-15 was assigned as equatorial from the ^{13}C chemical shift value (δ_C 30.5) compared to the value (δ_C 24.4) for the axial methyl group in **5**. Although such data should be used with care, the absence of an NOE correlation between Me-15 and H-6 was consistent with these stereochemical conclusions (Fig. 1). Isocyanide **1** has the same overall relative configuration as (1*S**,4*S**,6*S**,7*R**)-4-thiocyanate-9-cadinene **6** (δ_C 32.7 for Me-15) previously isolated by He et al.[25]. Accordingly, isocyanide **1** was characterized and named (1*S**,4*S**,6*S**,7*R**)-4-isocyano-9-amorphene.

3.2. Structure elucidation of a new 10-isocyano-4-amorphene diastereomer (2)

Initial ^{1}H NMR analysis of a fraction eluting from hexanes/DCM (4:1) indicated that it contained one major isonitrile component with a molecular formula of $C_{16}H_{25}N$ by HRESIMS measurement (*m/z*