

Natural Products from Three Nudibranchs: *Nembrotha kubaryana*, *Hypselodoris infucata* and *Chromodoris petechialis*

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Abstract: Nudibranchs are shell-less molluscs that are often brightly colored and seemingly defenseless against predation. However, these beautiful animals usually contain large amounts of diet-derived natural products that help defend them against predation. We have isolated a blue tetrapyrrole from *Nembrotha kubaryana*, the known nakafuran-8 and -9 from *Hypselodoris infucata* and spongiane-16-one from *Chromodoris petechialis*. These compounds have previously been found in other marine organisms, thus supporting a link between diet and natural products in the nudibranchs.

Keywords: Tetrapyrrole, spongiane, nakafuran, marine natural products.

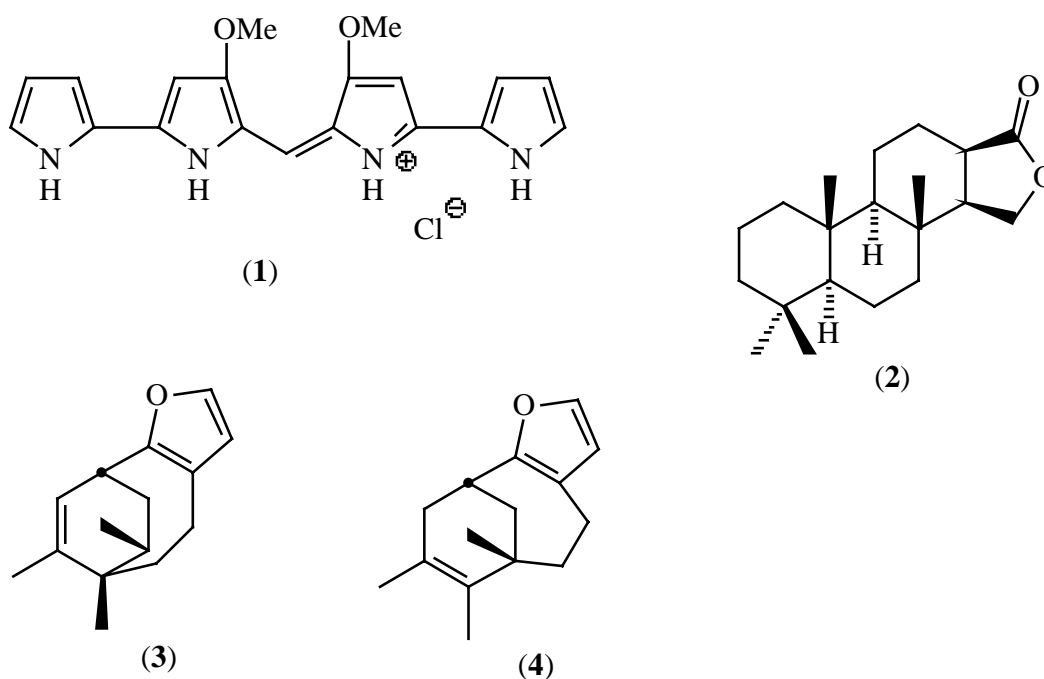
Introduction

It has long been recognized that nudibranchs are infrequently preyed upon even though they have no obvious morphological defense against predation [1]. In particular, the families Chromodoridae and Polyceridae are generally conspicuously colored and have invariably been found to contain compounds identical or similar to those found in their diet [2]. These compounds are accumulated on a functional basis, and it is possible that nudibranchs can be used by chemists to fractionate the best

chemical defense compounds from their diet. This biorationale approach suggests that the potent defense compounds may have other uses.

Results and Discussion

As part of our continuing study of natural products from marine invertebrates [3] we have isolated the known though unusual blue pigment **1** from the nudibranch *Nembrotha kubaryana* (Fig. 1A)[†]. This pigment has previously been isolated from a mutant strain of the bacterium *Serratia marcescens* [4], blue marine ascidians [5, 6] and a blue bryozoan [7]. Since nudibranchs of the family Nembrothidae are known to feed on either ascidians or bryozoans [8], presumably the metabolite is diet-derived.



The rather uncommon nudibranch, *Chromodoris petechialis* (Fig. 1B)[†], yielded three diterpenes. The major was identified as spongian-16-one (**2**) previously isolated from both New Zealand and Australian specimens of the sponge *Chelonaplysilla violacea* [9]. A species of *Chelonaplysilla* occurs in the nudibranch's habitat [10] and this may be the source of **2**. The same compound has also been isolated from another chromodorid nudibranch, *Chromodoris obsoleta* [11].

Extraction of the chromodorid nudibranch *Hypselodoris infucata* (Fig. 1C)[†] yielded a 3:1 mixture of nakafuran-8 (**3**) and nakafuran-9 (**4**) in the same ratio as found in the prey sponge, *Dysidea fragilis* [12]. Minor metabolites previously isolated from the sponge were however, not detected in the nudibranch extract.

†Figure 1 that appeared here in the original manuscript and available from the MDPI website from February 2002 to July 2003 was derived from previously copyrighted material. The authors wish to apologize to the Australian Museum for inadvertent infringement of their copyright. The deletion of this figure in no way affects the scientific content of the paper.

Compound **1** was found to be a potent antimicrobial agent; active against *B. subtilis* at 5 µg/disc. Nakafuran-8 and -9 were not found to be antimicrobial, though they have previously been reported as having fish antifeedant properties [12]. Spongian-16-one (**2**) has been reported as cytotoxic [11]. Too little material was available to perform antimicrobial assays.

Conclusions

Three nudibranchs that have not previously been chemically investigated yielded compounds which appear to be derived from their diets. The two chromodorids yield terpenoids potentially derived from their sponge diet and the nembrothid yielded a blue tetrapyrrole, most likely also derived from its ascidian diet.

These data support the dietary link between nudibranchs and their natural products. High concentrations of the compounds were found and it may be possible to use nudibranchs to indicate which compounds from their diet are most likely to be biologically active.

Experimental

General

All solvents were reagent grade and redistilled from appropriate drying agents before use. Analytical thin-layer chromatography (TLC) separations were carried out on Merck silica gel 60 F-254 (0.2 mm) precoated aluminum plates. Once developed, plates were visualized by spraying with 5% vanillin in sulfuric acid followed by gentle heating. High-performance liquid chromatography (HPLC) was done on a Waters 6000A solvent delivery system equipped with UV detection (254 nm). Normal phase separations were performed on an Alltech Adsorbosphere® 5 mm column (25 x 0.46 cm) and

reverse phase separations on an Adsorbosphere® HS 5 mm (25 x 0.46 cm) column. Nuclear magnetic resonance spectra (NMR) were recorded in CDCl₃ ("100%", Aldrich) on a GE-400 400MHz instrument using the solvent signal as internal standard. Electron impact mass spectrometry (EIMS) was performed on a MAT 311 instrument.

Sample Collection:

One specimen of *N. kubaryana* [8] was collected from the lagoon of Pohnpei in the Federated States of Micronesia. Seven specimens of *H. infucata* [13] were collected from Kaneohe Bay, Oahu, Hawaii and one specimen of *C. petechialis* [13] from Koko Head, Oahu.

Extraction and Isolation:

The frozen specimen of *N. kubaryana* was steeped in ethanol (10 mL) and subject to ultrasound for one hour. The deep blue solution was decanted and diluted with water, partitioned with ether and the ether layer chromatographed on silica (10% ethyl acetate/benzene). The blue fraction was further purified by reverse phase HPLC (95% methanol/ 5% 0.04M HCl) to yield pure **1** as blue crystals (1.5 mg; 0.5% wet weight); *m/z* 335 (M + H⁺; 50%), 334 (100); ¹H-NMR (400 MHz, CDCl₃) δ 7.16 (s, 2H), 6.87 (m, 2H), 6.40 (m, 2H), 6.12 (d, J = 2.6 Hz, 2H), 5.35 (s, 1H), 3.94 (s, 6H).

One specimen of *C. petechialis* was extracted in a similar fashion to *H. infucata* and the hexane extract filter-chromatographed on reverse phase silica (BondElute® 50-100% methanol/water). The fraction eluting with 100% methanol was subject to normal phase HPLC (10% ethyl acetate/hexane) to yield three compounds, the major being identified as **2** (0.5 mg; 0.2%), *m/z* 304 (M⁺, 10%), 289 (23), 192 (20), 191 (100); ¹H-NMR (400 MHz, CDCl₃) δ 4.2 (d, J = 10, 1H), 4.1 (dd, J = 10, 5.5 Hz, 1H), 2.55 (dd J = 8, 8 Hz, 1H), 2.3 (m, 1H), 2.1 (dd, J = 8, 5.5, 1H), 1.0-1.8 (m, ~12H), 0.86 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H), 0.77 (dd, J = 12, 2.5).

Seven fresh specimens of *H. infucata* were steeped in methanol overnight and the extract decanted. The hexane partition yielded a colorless oil with the characteristic odor of the nudibranch. HPLC on silica gel (1% ethyl acetate/hexane) yielded nakafuran-8 (37 mg; 1.3%); *m/z* 216 (M⁺, 10%), 201 (20), 174 (100); ¹H-NMR (400 MHz, CDCl₃) δ 7.12 (d, J = 2 Hz, 1H), 6.05 (dd, J = 2, 1 Hz, 1H), 5.95 (dd J = 7, 1 Hz, 1H), 3.46 (ddd, J = 7.5, 3.5, 1 Hz, 1H), 2.45 (ddd, J = 14, 4, 1 Hz, 1H), 2.26 (ddd, J = 14.5, 4, 1 Hz, 1H), 2.00 (ddd, J = 14.5, 4, 1 Hz, 1H), 1.91 (qdd, J = 7.5, 6 1 Hz, 1H), 1.81 (ddd, J = 14.5, 4, 1 Hz, 1H), 1.77 (d, J = 1 Hz, 3H), 1.29 (ddd, J = 14.5, 4, 1 Hz, 1H), 1.24 (ddd, J = 14.5, 4, 1 Hz 1H), 1.05 (s, 3H), 0.86 (d J = 7.5 Hz, 1H) and nakafuran-9 (11 mg; 0.4%); *m/z* 216 (M⁺, 20%), 201 (100); ¹H-NMR (400 MHz, CDCl₃) δ 7.16 (d, J = 2 Hz, 1H), 6.05 (dd, J = 2, 1 Hz, 1H), 3.16 (tddd, J = 4, 5.5, 2, 1 Hz, 1H), 2.35 (ddd, J = 14, 4, 1 Hz, 1H), 2.30 (dd, J = 14, 5.5 Hz, 1H), 2.27 (ddd, J = 14.5, 4, 1

Hz, 1H), 1.91 (ddd, J = 14, 1 1 Hz, 1H), 1.81 (ddd, J = 14, 4, 1 Hz, 1H), 1.77 (d, J = 4 Hz, 2H), 1.61 (d, J = 1 Hz, 3H), 1.60 (s, 3H), 1.35 (ddd, J = 14, 7, 1 Hz, 1H), 1.05 (s, 3H).

Bioassays:

Bioassays were conducted against *Esherichia coli* (NCTC 8196), *Staphylococcus aureus* (NCTC 4163), *Pseudomonas aeriginosa* (NCTC 6749) and *Bacillus subtilis* (NCTC 10400) using the disk diffusion assay of Bauer [14].

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Sample availability: Samples of the isolated compounds are not available

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