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Structure revision of the *Penicillium* alkaloids haenamindole and citreoindole

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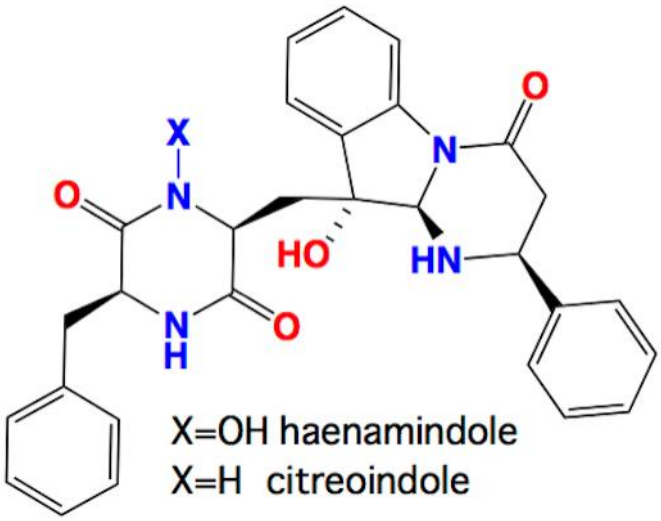
ABSTRACT

Herein, we describe the isolation of rare alkaloids, haenamindole and citreoindole, from a South China Sea deep-sea fungus, *Penicillium citrinum* (MF006), and their structure revision based on detailed spectroscopic and C_3 Marfey's analysis.

KEYWORDS: haenamindole; citreoindole; structure revision, deep-sea fungus

GRAPHICAL ABSTRACT



The genus *Penicillium* has been a prolific source of structurally diverse metabolites for over 90 years, with the antibiotic β-lactam penicillins, for example, heralding a revolution in the treatment of infectious disease. Notwithstanding past achievements, *Penicillium* isolates remain a valuable resource for microbial biodiscovery.

In our ongoing microbial biodiscovery program we used Dragendorff's reagent to screen a library of 946 marine-derived fungi for the presence of alkaloids, with an extract obtained from a South China Sea (-1500 m) sediment-derived *Penicillium citrinum* (MF006) exhibiting a positive response. As *P. citrinum* isolates have previously been reported to produce cytotoxic[1-3] and antibacterial[4-6] alkaloids, we were keen to investigate the alkaloids produced by MF006. A potato dextrose agar slant of MF006 was used to inoculate seed broth cultivations of (4 × 250 mL flasks, containing 40 mL of a liquid medium consisting of potato infusion (20%), glucose (2.0%), artificial sea salt (3.5%) and distilled water), which were incubated at 28 °C and 160 rpm for 3 d. Seed culture aliquots (5 mL) were used to inoculate larger cultivations (12 × 1 L flasks, each containing rice (100 g) and artificial seawater (3.5%; 30 mL)), which were incubated under static conditions at 25 °C for 21 d. The resulting rice-based cultivations were subjected to solvent extraction and reversed–phase HPLC fractionation to yield the known but otherwise rare alkaloids haenamindole (**1**)[7] and citreoindole (**2**).[8] Our re-investigation of **1** and **2** confirmed the need for structure revisions.

The HRESI(+)MS data for **1** revealed a sodium adduct ion consistent with a molecular formula ($C_{29}H_{28}N_4O_5$, Δmmu +0.6) requiring 18 double bond equivalents (DBE). The 1D NMR (DMSO-d_6) data for **1** (ESI) revealed resonances for two mono-substituted and an *ortho*-disubstituted benzene ring, as well as three ester/amide carbonyls (δ_C 166.1, 159.8 and 168.8), accounting for

15 DBE and requiring that **1** be hexacyclic. The NMR data for **1** (ESI, Table S1) proved to be identical with that for haenamindole (**1a**) ($[\alpha]_D$ –86.4, *c* 0.3, MeOH), first reported in 2015 by Kim and co-workers from a marine sediment-derived *Penicillium* sp. KCB12F005 ($[\alpha]_D$ –89.4, *c* 0.05, MeOH).[7] A C_3 Marfey's analysis[9] on **1** confirmed the presence of L-Phe and L-β-Phe residues, with a diagnostic ROESY correlation from H-10 to H-12 supporting the 10*R*, 12*S* and 24*S* configuration as previously noted by Kim and co-workers for **1a**.

We were however skeptical about the configurational assignment made by Kim and co-workers, where the absence of ROESY correlations between H-21 and H-24, and H-10 and 9-OH, was taken as evidence of *trans* relationships, and hence a 9*S*, 21*R* configuration. Interpretations based on the absence of ROESY/NOESY correlations should be approached with great caution. In our hands we observed ROESY correlations between H-21 and H-24, and H-10 and 9-OH, consistent with *cis* relationships and a 9*R*, 21*S* configuration. Based on these observations we propose the revised structure for haenamindole (**1**) as indicated (Figure 1).

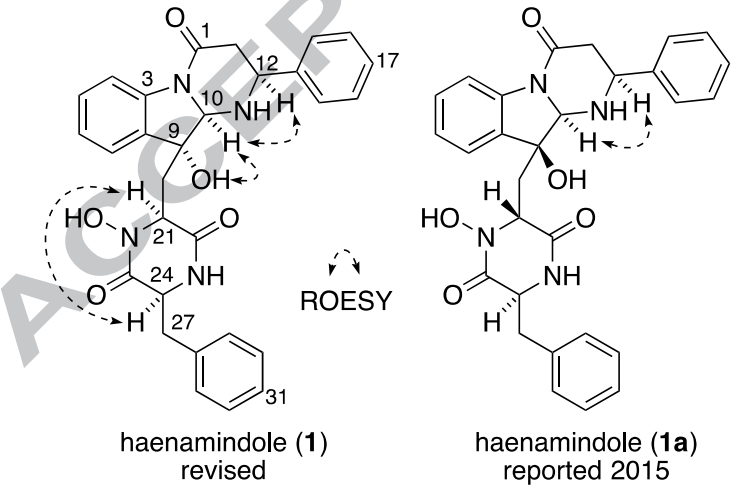
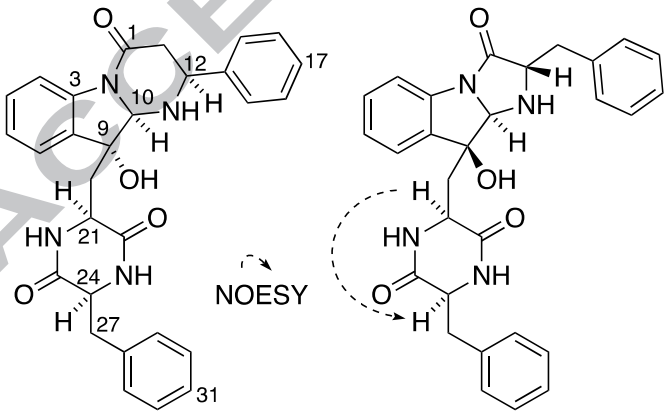


Figure 1. Revised (**1**) and reported (**1a**) structures for haenamindole

The HRESI(+)MS data for **2** revealed a sodium adduct ion consistent with a molecular formula ($C_{29}H_{28}N_4O_4$, Δmmu +0.0) for a deoxy analogue of **1**. On reviewing the literature, the NMR (DMSO-d_6) data for **2** (ESI, Table S2) proved identical with that for citreoindole (**2a**) ($[\alpha]_D$ – 38.1, *c* 0.06, MeOH), first reported in 1991 by Matsunaga and co-workers from a hybrid strain derived from *Penicillium citreo-viride* B. IFO 6200 and 4692 (no reported $[\alpha]_D$).[8] Matsunaga and co-workers used chiral HPLC on a hydrolysate of **2a** to detect only L-Phe. By contrast, we employed C_3 Marfey's methodology[9] to identify both L-Phe and L-β-Phe residues in **2**, consistent with the biosynthetically related co-metabolite **1**.

While we accepted the H-21 to H-24 NOESY correlation observed by Matsunaga and co-workers, and the associated 21*S* assignment, by misidentifying the L-β-Phe residue we had concerns regarding other conformational assignments. Based on the above, as well as spectroscopic and biogenetic considerations, we propose that **1** and **2** possess the same absolute configuration, and attribute the revised structure for citreoindole (**2**) as indicated (Figure 2).



citreoindole (**2**) revised

citreoindole (**2a**) reported 1991

Figure 2. Revised (**2**) and reported (**2a**) structures for citreoindole

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SUPPORTING INFORMATION

General experimental conditions, including fungal taxonomy and cultivations, and the extraction, isolation and characterization of **1** and **2**, as well as tabulated 1D and 2D NMR data, and selected spectra. This material is available free of charge via the Internet at http://pubs.acs.org

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Highlights

Deep-sea

Fungus

Structure revision

Haenamindole

Citreoindole