Secondary metabolites from a marine sponge *Cliona patera*

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Received 7 November 2007; accepted 25 January 2008

**Keywords:** Marine sponge; *Cliona patera*; Tetillapyrone; Nortetillapyrone; Maleimide 5-oxime; 3-Methylmaleimide-5-oxime; cyclo-(t-Pro-l-Tyr)

1. Subject and source

*Cliona patera* (Hardwicke, 1822), order Hadromerida, family Clionidae, was collected by scuba diving in the Gulf of Thailand near Koh Lan at 18 m depth, Chonburi Province, Eastern Thailand, in May 2004 and frozen immediately at −20°C prior to extraction. The sponge was identified by Dr. Sumaitt Putchatkarn. A voucher registered as BIMS-I 1465 is deposited in the Bangsaen Institute of Marine Science, Burapha University, Thailand. This burrowing sponge, earlier found only in Indonesian and Australian waters, was thought to have been extinct since 1912 (R. van Soest, private communication) but has recently been discovered in the Gulf of Thailand at more than 15 m depth (Putchatkarn, 2005).

2. Previous studies

Earlier studies of *Cliona species* described various sterols from *Cliona caribbaea* (Bergmann et al., 1950), *Cliona celata* (Erdman and Thomson, 1972), *Cliona viridis* (Sica et al., 1978), *Cliona chilensis* (Rovirosa et al., 1984) and the sesquiterpene plumericin from *C. caribbaea* (Martin et al., 1985). A tryptophan derivative clionamide (Andersen, 1978; Andersen and Stonard, 1979) and four more complex peptide alkaloids celenamides

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A–D were isolated from C. celata (Stonard and Andersen, 1980a,b) and celenamide E, a tryptophan-based tripeptide alkaloid, was reported from the Patagonian sponge C. chilensis (Palermo et al., 1998) while the methanol extract of Cliona delitrix from the Gulf of Santos, Brazil which exhibited antimitotic activity against the tumor cell line MCF-7 gave as the only significant product 5-hydroxytryptophan (Granato et al., 2006). Structures of pyrostatins A and B, inhibitors of N-acetyl-β-D-glucosaminidase produced by a Streptomyces species, have been revised as a consequence of their discovery in Cliona tenuis (Castellanos et al., 2006), an urea derivative acetyl-d-glucosaminidase has been found in C. celata collected on the coast of Galicia (Lenis et al., 1996) and four novel peptide alkaloids, storniamides A–D, based on pyrrole have been isolated from the ethanol extract of an unnamed Cliona species collected near Punta Verde, Argentina (Palermo et al., 1996). A series of 3β,5α,6β-trihydroxylated sterols (Notaro et al., 1991), steroidal hydroxyketones and closely related diols (Notaro et al., 1992) were isolated from Cliona copiosa collected in the Bay of Naples, while Cliona nigricans afforded two polychlorinated androstanes which were active against three tumor cell lines (Fattorusso et al., 2004).

3. Present study

3.1. General procedures

1H and 13C NMR spectra were recorded at room temperature in CDCl3 on a Bruker AMC instrument operating at 300.12 and 75.47 MHz, respectively, or a Bruker DRX instrument operating at 500 and 125 MHz. EI mass spectra were measured on a Hitachi Perkin–Elmer RMV-6M instrument. HR spectra were measured on a Kratos concept II 2 sector mass spectrometer. The accelerating voltage was 8 kV. Melting points were recorded on a Bock monoscope and are uncorrected. Rotations were determined on a Polax-2L instrument. Si gel for chromatography was silica gel 60 (0.2–0.5 mm Merck) for analytical work and for preparative work TLC silica gel 60 GF 254 Merck.

3.2. Extraction, isolation and characterization of the constituents

The freeze-dried sponge (12 kg net weight) was thawed, homogenized with EtOH (10 L), allowed to stand for 24 h in a dark chamber and filtered. The residue on the filter paper was again extracted with EtOH (2 × 10 L); the aqueous alcoholic extracts were combined and evaporated at reduced pressure to give 12 g of the crude EtOAc extract. This extract was chromatographed over a Si gel column (60 g) and eluted with petrol–CHCl3 and CHCl3–Me2O to give 150 ml fractions as follows: Frs 1–17 (petrol–CHCl3, 1:1), 18–50 (petrol–CHCl3, 3:7), 51–60 (petrol–CHCl3, 1:9), 61–94 (CHCl3–Me2O, 9:1), 95–124 (CHCl3–Me2O, 4:1), 125–134 (CHCl3–Me2O, 3:2), 135–150 (CHCl3–Me2O, 1:9). Frs 9 and 10 (860 mg) were combined and recrystallized from a mixture of CHCl3 and MeOH to give 537 mg of clionasterol. Frs 28–46 (432 mg) were combined and recrystallized from a mixture of CHCl3 and MeOH to give 373 mg of a mixture of glycerol ethers 4a and 4b. Frs 72–78 (136 mg) were combined and purified by preparative TLC (Si gel CHCl3–MeOH–HCO2H, 9:1:0.1) to give 23 mg of a mixture of p-hydroxybenzoic acid and cyclo-[(L-Pro-L-Tyr)] (1) followed by 34 mg of 4a. Compound 4a has been previously found in the sponge Pseudoceratina purpurea (Kijjoa et al., 2005). The mixture of p-hydroxybenzoic acid and 1 was submitted to preparative TLC (Si gel CHCl3–MeOH–HCO2H, 85:15:0.1) to afford 6 mg of the acid and 12 mg of 1.

Frs 91–95 (45 mg) of the original chromatogram on cristallization from a mixture of CHCl3 and petroleum ether gave 32 mg of previously unreported maleimide 5-oxime (4b). Frs 107–111 were combined (21 mg) and purified by preparative TLC (Si gel, CHCl3–MeOH–HCO2H, 85:15:0.1) to give 12 mg of tetillapyrone (2) (Wattanadilok et al., 2001) while combination of Frs 121–134 (134 mg) and purification by TLC in the same fashion afforded an additional 43 mg of 2 and 32 mg of nortetillapyrone (3). Known compounds 1, 2, 3 and 4a were identified by HRMS, 1H and 13C NMR spectrometry.

Maleimide 5-oxime (4b) was isolated as a yellow gum; EI HRMS 112.02723 (M+), calc for C6H4N2O2 112.02728; 1H NMR (300 MHz, DMSO-d6), δ 11.03 br s (−NH), δ 10.86 br s (=N−OH), 7.40 d (J = 7.6 Hz, H-4), 5.45 d (J = 7.6 Hz, H-3), 13C NMR (75.47 MHz, DMSO), δ 164.37 (CO-2), 151.54 (C-5), 142.22 (CH-4), 100.24 (CH-3).
Glycerol ethers $5a$ and $5c$ have been prepared repeatedly, initially by Wood and Snyder (1967) who reported their $^1$H NMR spectra, but glycerol ether $5b$ is new and $^{13}$C NMR spectra of $5a$ and $5c$ have not appeared previously in the literature. The mixture of glycerol ethers $5a$–$c$ was isolated as a gum; FAB MS 345, 331, 317; FAB HRMS 345.33692, 331.32118, 317.30560 (M + H), cld for C$_{21}$H$_{45}$O$_3$ 345.33687, cld for C$_{20}$H$_{43}$O$_3$ 331.32122, cld for C$_{19}$H$_{41}$O$_3$ 317.30560. $^1$H NMR (300 MHz, CDCl$_3$), $\delta$ 3.68 dd ($J = 11.5, 5.8$ Hz, H-1a), 3.58 dd ($J = 11.5, 3.6$ Hz, H-1b), 3.85 q (5.7 Hz, H-2), 3.46 d (3.8 Hz, H-3a, b), 3.44 t (6.7 Hz, H-10), 1.53 q (6.5 Hz, H-20), 1.24 br s (24 H, H-30, H-14), 0.86 t (6.5 Hz, CH$_3$-15), 3.31 br s (OH), $^{13}$C NMR (CDCl$_3$, 75.47 MHz), $\delta$ 72.20 C-3, 71.75 C-10, 70.63 C-2, 64.05 C-1, 31.86 C-13, 29.64 CH$_2$, 29.61 CH$_2$, 29.58 CH$_2$, 29.56 CH$_2$-2, 29.49 CH$_2$, 29.44 CH$_2$, 29.31 CH$_2$, 25.99 CH$_3$-2, 22.63 CH$_2$-14, 14.06 CH$_3$-15. Acetylation in the usual manner afforded a mixture of the diacetates; $^1$H NMR spectrum $\delta$ 4.34 dd ($J = 12.0$, 3.7 Hz, H-1a), 4.16 dd ($J = 12.0$, 6.3 Hz, H-1b) 5.18 m (H-2), 3.55 d ($J = 5.2$ Hz, H-3), 3.73–3.47 m (H-10), 1.55 q ($J = 6.8$ Hz, H-20) 1.25 br s (H-30–H-15), 0.88 t ($J = 6.5$ Hz, terminal Me) $^{13}$C NMR $\delta$ 170.73 (Ac CO), 170.40 (Ac CO), 71.73 (C-10), 70.29 (C-2), 68.60 (C-3), 62.97 (C-1), 31.91, 39.68, 39.61, 29.58, 29.48, 29.43, 29.35, 29.58, 22.68 (CH$_2$-2–15), 21.05 and 20.78 (acetate Me’s), 14.13 (Me).

4. Chemotaxonomic significance

The diversity of secondary metabolites reported so far from this genus allows for few, if any, generalizations although tryptophan derivatives are relatively frequent. The source of 1, a relatively common fungal metabolite
(Park et al., 2006), is probably a fungus associated with *C. patera* as in the case of the sponge *Jaspis digonoxea* (Rudi et al., 1994) and other sponges (e.g. Jayatilaka et al., 1996; DeRosa et al., 2004). Very recently tetillapyrone (2) and nortetillapyrone (3), originally isolated from the sponge *Tetilla japonica* (family Tetillidae, order Spirophorida) (Wattanadilok et al., 2001), have also been found in *Haliclona cymbiformis* and *Haliclona baeri* (family Haliclonaidea, order Haplosclerida) (Wattanadilok et al., 2007) all collected, as was *C. patera*, in shallow waters in the Gulf of Thailand. These isolations of 2 and 3 from widely different taxa may suggest an extraneous source of these compounds as they have so far not been found in other representatives of these genera.

**Acknowledgment**

Work in Portugal was supported by FTC — Fundação para a Ciência e Tecnologia (Project POCI/MAR/58114/2004). We thank Dr. Sumaitt Putchatkarn, BIMS, Burapha University, Thailand, for collection and identification of sponge material.

**References**


