Antioxidant Alkaloid from the South China Sea Marine Sponge *Iotrochota* sp.

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Purpurone was isolated from the sponge *Iotrochota* sp. by bioactivity-guided fractionation. The compound showed antioxidant activity using the DPPH assay. The structure was established on the basis of NMR data and comparison with data reported.

Key words: Marine Sponge, *Iotrochota*, Purpurone

Introduction

Marine sponges belonging to the genus *Iotrochota* are a promising source of diverse chemical metabolites with a wide range of bioactivity. Studies on the marine sponge *Iotrochota* sp. resulted in the bromoindole methyl (*E*)-3-(6-bromoindol-3-yl) prop-2-enoate (Dellar *et al*., 1981) and the aromatic pyrrole derivative purpurone (**1**) (Chan *et al*., 1993). Three halogenated tyrosine derivatives (Costantino *et al*., 1994), five ecdysteroids (Costantino *et al*., 2000), and five sterols with a nucleus skeleton of 6-hydroxy-4-en-3-one (Li *et al*., 2005) have been isolated from *Iotrocbota birotulata*. Itampolins A and B (Sorek *et al*., 2006), cytotoxic and antibacterial matemone and 6-bromoindole-3-carbaldehyde (Carletti *et al*., 2000) were reported from *Iotrochota purpurea*. The cytotoxic glycosphingolipid iotroridoside A (Deng *et al*., 2001), 3-octadecyloxy-1,2-propanediol (batyl alcohol) (Tian and Deng, 1998), and two ceramides (Liang *et al*., 2000) have been isolated from *Iotrochota ridley*. In addition, the sponge *Iotrochota baculifera* was reported to contain six sphingolipids and the glycosphingolipid iotroridoside B (Muralidhar *et al*., 2003, Muralidhar and Rao, 2006).

In our investigation, the aromatic alkaloid **1** was isolated as a purple solid from the sponge *Iotrochota* sp. Compound **1** was identified by comparison of its spectral data with those of purpurone, which was isolated from the same genus. Purpurone inhibited ATP-citrate lyase in a dose-dependent manner and had an IC_{50} value of 7 μM (Chan *et al*., 1993).

Results and Discussion

In the course of our search for antioxidants in marine sponges, we detected in the ethanolic extract of the marine sponge *Iotrochota* sp. purple spots, that reduced the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in a TLC autographic assay (IC_{50} 183.97 μg/ml). Compound **1** was evaluated for antioxidant activity using the DPPH assay, and proved to possess potent scavenging activity (IC_{50} 19 μM). The results presented here indicate a high content of the aromatic purple pigment alkaloid in the marine sponge *Iotrochota* sp., which constitute its active antioxidant principle.

To our best knowledge, the purple alkaloid purpurone (**1**, Fig. 1) was reported for the first time from the marine sponge *Iotrochota* collected in September 1983 at the Koror Island, Palau. The purple active component was obtained from a hydrolyzed fraction of the ethanolic extract. It was therefore speculated to originate from precursors which were either sugar or protein conjugates (Chan *et al*., 1993). Our investigations on *Iotrochota* obtained from South China Sea also resulted in purpurone as a natural product. Consistent, reliable production and the ease, with which this purple pigment spot can be visualized without using

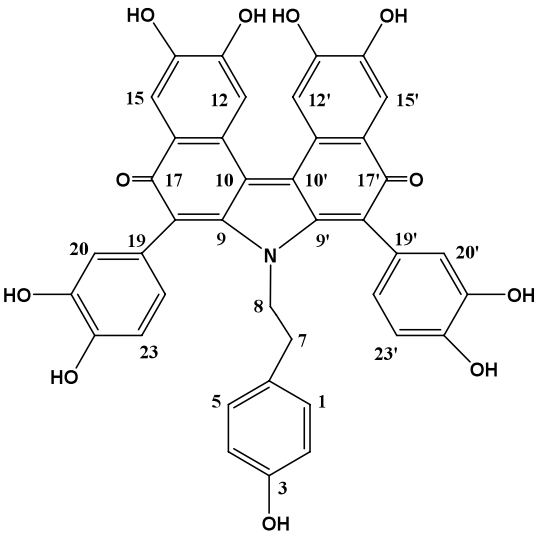

Fig. 1. Chemical structure of purpurone (**1**).

any reagent during TLC profiling, make it an important chemotaxonomic marker for *Iotrochota* sp. Species of the genus *Iotrochota* are frequently difficult to differentiate due to their morphological characteristics. Thus, multidisciplinary approaches that combine histological, ecological, and/or chemical data with sponge morphology have proven useful to differentiate between closely related species.

Experimental

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-500 spectrometer. Chemical shifts were reported with reference to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). Reverse-phase HPLC was performed on a semipreparative YMC C18 column (250 × 5 mm, 5 μm), using a Hitachi 2000 UV-VIS detector.

Animal material

The sponge was collected in August 2005 (5–6 m depth), off the coast of Hainan Island, China. The specimen was identified as *Iotrochota* sp. by Dr. Kyung Jin Lee, Invertebrate Research Division, National Institute of Biological Resources, Environmental Research Complex, Incheon, Korea. A voucher specimen of the sponge (No. 20050801) was deposited at the Natural History Museum, Hannam University, Daejon, Korea and Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Guangzhou, China.

Extraction and isolation

The sponge (wet weight 10 kg) was extracted three times with EtOH at room temperature. The EtOH extract was partitioned between water and $CHCl_3$. The $CHCl_3$ layer was further partitioned between 80% EtOH and *n*-hexane to yield 80% EtOH- (80 g) and *n*-hexane-soluble (70 g) fractions. The water layer was further extracted with *n*-BuOH to give the residue (35 g). These fractions were evaluated for DPPH activity; the *n*-BuOH fraction was found most active (IC_{50} values for EtOH, *n*-hexane, *n*-BuOH fractions were 540.4, 781.4, and 139.6 μg/ml, respectively). Guided by the DPPH assay, the *n*-BuOH extract was subsequently subjected to reverse-phase column chromatography, eluting with a solvent system of 10–95% EtOH/H_2O, to afford 18 fractions (Fg1–Fg18). Fraction Fg11 was separated by semipreparative ODS HPLC, eluting with 30% MeOH/H_2O, to afford compound **1** (8.5 mg), a stable purple solid when kept in a freezer.

Purpurone (**1**): Purple solid. – ^{1}H NMR (500 MHz, CH_3OD): δ = 7.86 (2H, *s*, H-12, 12′), 7.47 (2H, *s*, H-15, 15′), 6.84 (2H, *d*, *J* = 1.6 Hz, H-20, 20′), 6.83 (2H, *d*, *J* = 9.2 Hz, H-23, 23′), 6.69 (2H, *dd*, J_1 = 8.0 Hz, J_2 = 1.6 Hz, H-24, 24′), 6.46 (2H, *d*, *J* = 8.4 Hz, H-1, 5), 6.40 (2H, *d*, *J* = 8.4 Hz, H-2, 4), 2.99 (2H, *t*, *J* = 7.0 Hz, H-8), 2.21 (2H, *t*, *J* = 7.0 Hz, H-7). – ^{13}C NMR (125 MHz, CH_3OD): δ = 130.9 (C-1, 5), 115.6 (C-2, 4), 156.7 (C-3), 129.7 (C-6), 34.6 (C-7), 47.7 (C-8), 156.1 (C-9, 9′), 126.4 (C-10, 10′), 125.0 (C-11, 11′), 114.1 (C-12, 12′), 150.3 (C-13, 13′), 149.1 (C-14, 14′), 115.0 (C-15, 15′), 132.2 (C-16, 16′), 185.4 (C-17, 17′), 118.9 (C-18, 18′), 126.1 (C-19, 19′), 119.4 (C-20, 20′), 146.2 (C-21, 21′), 146.6 (C-22, 22′), 116.2 (C-23, 23′), 124.1 (C-24, 24′).

DPPH assay

DPPH scavenging activity was measured according to the procedure described by Blois (1958) and Braham *et al.* (2005). Briefly, each test sample (50 μl) of various concentrations (0.015–2.0 mg/ml) was added to 950 μl of freshly prepared DPPH solution (0.004% in MeOH), and the mixture was

vortexed for 15 s. The decrease in absorbance at room temperature was determined at 515 nm after 30–45 min of incubation, until the reaction reached a steady state. All experiments were performed in triplicate. The inhibition percentage (%) of radical scavenging activity was calculated according to: $(1 - A_S/A_0) \cdot 100$, where A_0 and A_S are the absorbance of the control and the sample, respectively, at 515 nm. α-Tocopherol and quercetin were used as standards.

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