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

Yonghong Liu\(^a,\ast\), Hong Ji\(^a\), Junde Dong\(^a,b\), Si Zhang\(^a\), Kyung Jin Lee\(^c\), and Susan Matthew\(^d\)

\(^a\) Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510–301, China. Fax: +86-20-8445 1672. E-mail: yonghongliu@scsio.ac.cn

\(^b\) National Experiment Station of Tropical Marine Biology, Sanya 572–000, China

\(^c\) Invertebrate Research Division, National Institute of Biological Resources, Environmental Research Complex, Incheon 404–170, Korea

\(^d\) Department of Medicinal Chemistry, Health Science Center, University of Florida, Gainesville, USA

* Author for correspondence and reprint requests

Z. Naturforsch. **63**c, 636–638 (2008); received February 11/March 17, 2008

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 *Iotrochota 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 (betyl 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 \(\mu\)m (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 \(\mu\)g/ml). Compound 1 was evaluated for antioxidant activity using the DPPH assay, and proved to possess potent scavenging activity (IC\(_{50}\) 19 \(\mu\)m). 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 of the marine sponge *Iotrochota* 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...
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**

1H and 13C NMR spectra were recorded on a Bruker AC-500 spectrometer. Chemical shifts were reported with reference to the respective residual solvent peaks (δH 3.30 and δC 49.0 for CD3OD). 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 Material 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 CHCl3. The CHCl3 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 (IC50 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/H2O, to afford 18 fractions (Fg1–Fg18). Fraction Fg11 was separated by semipreparative ODS HPLC, eluting with 30% MeOH/H2O, to afford *Purpurone* (8.5 mg), a stable purple solid when kept in a freezer.

**Purpurone** (1): Purple solid.  
- 1H NMR (500 MHz, CD3OD): δ = 7.86 (2H, s, H-12, 12/H11032), 7.47 (2H, s, H-15, 15/H11032), 6.84 (2H, d, J = 1.6 Hz, H-20, 20/H11032), 6.83 (2H, d, J = 9.2 Hz, H-23, 23/H11032), 6.69 (2H, dd, J1 = 8.0 Hz, J2 = 1.6 Hz, H-24, 24/H11032), 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).  
- 13C NMR (125 MHz, CD3OD): 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

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (No. 40706046), Knowledge Innovation Program of Chinese Academy of Sciences (LYQY200703), and Guangdong Key Laboratory of Marine Mater-ria Medica Foundation.