A new compound was isolated from the water-soluble fraction of a methyl alcohol extract obtained from the larva of the dobsonfly (Protohermes grandis Thunberg). The novel compound had a 12-membered ring and was confirmed to be a cyclic dipeptide of d-ornithine by a chiral analysis, using high-performance liquid chromatography, 2D-nuclear magnetic resonance, and field desorption mass spectrometry.

Key words: cyclic di-D-ornithinate; dobsonfly; larva; Protohermes grandis

We have recently been searching for novel insect ingredients to be used as crude drugs in traditional East Asian medicine. Larvae of the dobsonfly, Protohermes grandis Thunberg (Neuroptera, Corydalidae), an aquatic insect, have been prescribed as a crude drug in traditional Japanese medicine (called magotaro mushi in Japanese). Previously characterized constituents of the larvae included essential amino acids, fats, and some steroids; however, other low-molecular-weight polar constituents of the larvae have not been investigated.

Air-dried larvae of Protohermes grandis Thunberg (from the Sai river in the Shiroishi district of Iwate Prefecture, Japan) were purchased from Honzoukaku (from the Sai river in the Shiroishi district of Iwate Prefecture, Japan) were purchased from Honzoukaku (Nagoya, Japan). After a 40-d incubation period, the larvae (24.5 g) were soaked in methanol (2 liters) at room temperature and filtered. The filtrate gave a brown extract (6.4 g). This extract was separated into two portions, and CHCl₃, MeOH, and H₂O were added in a final ratio of 1:2:1.5. Each solution was shaken, and the upper (methanol-water) layer was isolated and concentrated in vacuo until dry, yielding the polar fraction (1820 mg) was separated into four fractions by open-column chromatography (Sephadex LH-20, Pharmacia Biotech., 45 mm I.D. × 450 mm; eluent, 50% MeOH/H₂O → MeOH → EtOAc). The eluted H₂O fraction (1820 mg) was separated into four fractions by open-column chromatography (Sephadex LH-20, Pharmacia Biotech., 45 mm I.D. × 450 mm; eluent, 50% MeOH/H₂O). The first-eluted fraction (1030 mg) of the previous separation contained trehalose, pyroglutamate, and compound 1. Finally, the compounds were refined by repeated high-performance liquid chromatography (HPLC; Sugar-D, Nacalai Tesque, 4.6 mm I.D. × 250 mm; eluent, 85% CH₃CN/H₂O) which yielded 2.5, 3.0, and 1.0 mg, respectively.

Compound 1 [λmax (KBr) cm⁻¹: 1559 (HN–C=O), 1698 (C=O)] was a white powder with a positive optical rotation, [α]D²⁵ +9.1° (c 0.1, H₂O). The ¹H-NMR and ¹³C-NMR spectra (in D₂O) of compound 1 indicated the presence of one methine group [Cb–H: δC 64.0, δH 4.13 (dd, J = 7.6, 6.1 Hz)], three methylene groups [Cc–H: δC 31.7, δH 2.08 (1H, m), 2.34 (1H, m); Cd–H: δC 26.5, δH 2.02 (2H, m); Ce–H: δC 48.9, δH 3.35 (1H, m), 3.44 (1H, m)], and one carbonyl (or carboxyl) carbon (Ca: δC 177.0).

The ¹H-¹H COSY, HMBC (J constant = 145 Hz), and HMBC (long-range J constant = 8 Hz) spectra of compound 1 indicated connectivity of the O=Ca–CbH–CbH–CeH₂–CeH₂–NH–molecules. The ¹H-NMR chemical shift of Cb–H suggested that a polar functional group (–NH₂) was linked to carbon Ca.

Compound 1 produced field desorption mass spectrometric (FD-MS) ion peaks at m/z 227 (8), 211 (30), and 195 (100) which were attributable to the [M – H]⁺, [227 – NH₂]⁺, and [211 – NH₂]⁺ ions for the molecular-related positive ions (hydride-fragment ions), respectively (Fig. 1). The negative ion electrospray ionization mass spectrum (ESI-MS) contained a [M – H]⁻ ion peak at m/z 227 (5), and [M – 2H]⁻ ion peak at m/z 113 (18). The high-resolution (HR) FD-MS data indicated the molecular ion composition, C₁₀H₁₉N₂O₂, for the [M – H]⁺ ion (227.1503, calculated for C₁₀H₁₉N₂O₂: 227.1508).

Taken together, these results suggest that compound 1 had a 12-membered ring and was a cyclic dipeptide of ornithine.

Fig. 1. Part of the FD-MS Data (carbon emitter) Spectrum of Compound 1.
The product (1a, FD-MS, \( m/z = 133 \) (40) \([M+H]^+\) and 132 (3) \([M]^+\); neg. ion-FAB MS, glycerol matrix, \( m/z = 149 \) (40) \([M+H_2O-H]^−\)) of the acid hydrolysate (3N HCl, 100°C, 4 h) of compound 1 (0.4 mg) yielded a single peak (\( R_t = 3.54 \) min) after an HPLC analysis (Chiralpak CR(+), 4.6 mm I.D. × 150 mm; solvent, H₂O; pH adjusted to 1.5 with HClO₄; flow rate, 0.4 ml/min; detection, 210 nm).

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This is the first report of this newly identified compound from a natural material. One of the antibiotics, \( \beta \)-lactams and macrolides that have a lactone structure are a group of compounds which have strong characteristic bioactivity. Since compound 1 had a lactam structure with a 12-membered ring, its physiological significance is of interest.

**Instruments.** The optical rotation of compound 1 was measured at 25°C with a polarimeter (P-1020; Jasco, Tokyo, Japan). Mass spectra (MS) and HR-MS were recorded by a JMX-HX 110 spectrometer (Jeol). The NMR spectra were recorded by JNM-GSX 400 and JNM-ECA 600 spectrometers (Jeol). HPLC was performed by using 510 pump (Waters, Milford, MA, USA), U-620 column oven (Sugai, Tokyo, Japan), UV-visible detector (Jasco), 504R RI detector (GL Sciences, Tokyo, Japan), and C-R-8A integrator (Shimadzu, Kyoto, Japan). The Chiralpak CR(+) HPLC column was obtained from Daicel (Hiroshima, Japan), the Sumichiral OA-6100 column was obtained from Sumika Chemical Analysis Service (Osaka, Japan), and the Sugar-D column was purchased from Nacalai Tesque (Kyoto, Japan).

**Chemicals.** D- and L-ornithine (special grade; Wako, Osaka, Japan) were obtained commercially.

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**References**