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Note

Cyclic Dipeptide of D-Ornithine Obtained from the Dobsonfly, *Protohermes grandis* Thunberg

Ryuichiro TANAKA† and Masashi ODA

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan

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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.[1)] 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;[2,3)] 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 (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 $CHCl_3$, MeOH, and H_2O were added in a final ratio of 1:2:1.[4)] Each solution was shaken, and the upper (methanol-water) layer was isolated and concentrated *in vacuo* until dry, yielding the polar fraction sample (3.1 g). This sample was subjected to open-column chromatography (Diaion HP-20, Mitsubishi Chemical, 40 mm I.D. × 175 mm; eluent, $H_2O \rightarrow$ 50% MeOH/$H_2O \rightarrow$ MeOH $\rightarrow$ EtOAc). The eluted H_2O 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/H_2O). 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% CH_3CN/H_2O) which yielded 2.5, 3.0, and 1.0 mg, respectively.

Compound **1** [ν_{max} (KBr) cm^{-1}: 1559 (HN–C=O), 1698 (C=O)] was a white powder with a positive optical rotation, $[\alpha]^{25}_D$ +9.1° (*c* 0.1, H_2O). The ^{1}H-NMR and ^{13}C-NMR spectra (in D_2O) of compound **1** indicated the presence of one methine group [Cb–H: δ_C 64.0, δ_H 4.13 (dd, J = 7.6, 6.1 Hz)], three methylene groups [Cc–H_2: δ_C 31.7, δ_H 2.08 (1H, m), 2.34 (1H, m); Cd–H_2: δ_C 26.5, δ_H 2.02 (2H, m); Ce–H_2: δ_C 48.9, δ_H 3.35 (1H, m), 3.44 (1H, m)], and one carbonyl (or carboxyl) carbon (Ca: δ_C 177.0).

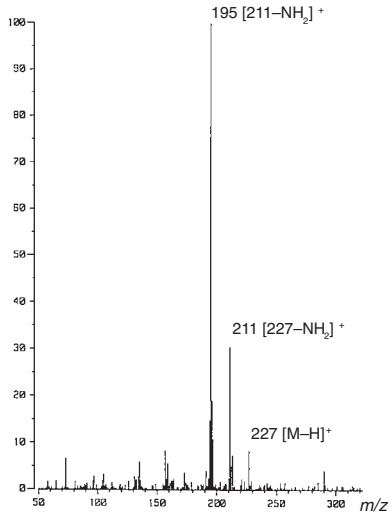

Fig. 1. Part of the FD-MS Data (carbon emitter) Spectrum of Compound **1**.

The ^{1}H-^{1}H COSY, HMQC (J constant = 145 Hz), and HMBC (long-range J constant = 8 Hz) spectra of compound **1** indicated connectivity of the O=Ca–CbH–CcH_2–CdH_2–CeH_2–NH–molecules. The ^{1}H-NMR chemical shift of Cb–H suggested that a polar functional group (–NH_2) 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 $[M - H]^+$, $[227 - NH_2]^+$, and $[211 - NH_2]^+$ 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 $[M - 2H]^{2-}$ ion peak at m/z 113 (18). The high-resolution (HR) FD-MS data indicated the molecular ion composition, $C_{10}H_{19}N_4O_2$, for the $[M - H]^+$ ion (227.1503, calculated for $C_{10}H_{19}N_4O_2$: 227.1508).

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

† To whom correspondence should be addressed. Tel/Fax: +81-72-866-3139; E-mail: tanaka-r@pharm.setsunan.ac.jp

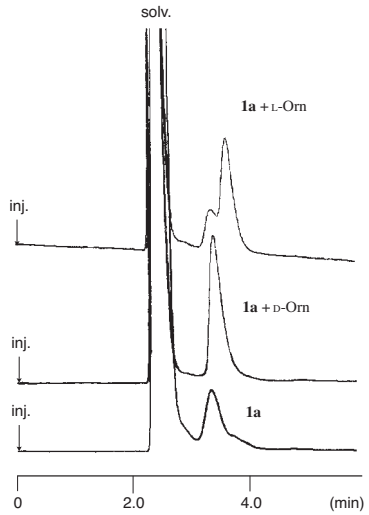

Fig. 2. HPLC Profiles of the Acid Hydrolysis Product of Compound **1**.

Column, Chiralpak CR(+), 4.6 mm I.D. × 150 mm; solvent, H_2O, pH adjusted to 1.5 with $HClO_4$; flow rate, 0.4 ml/min; detection, 210 nm

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 (3 N 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, H_2O, pH adjusted with $HClO_4$ at 1.5; flow rate, 0.4 ml/min; detection, 210 nm; *R*t values: D-Orn, 3.54 min; L-Orn, 3.84 min). The co-HPLC chromatography suggested that the product from acid hydrolysis was a D-isomer (Fig. 2). Furthermore, the chiral HPLC analysis of the hydrolysate using a Sumichiral OA-6100 column (4.6 mm I.D. × 250 mm, solvent of 1.5 mM copper (II) sulfate in H_2O, flow rate of 1.0 ml/min, detection at 254 nm, *R*t values: D-Orn, 8.28 min; L-Orn, 16.76 min) also indicated the presence of D-ornithine.

Two-dimensional nuclear magnetic resonance (2D-NMR), FD-MS, and chemical data indicated that the structure of compound **1** was (3*R*,9*R*)-2,8-dioxo-1,7-diazacyclododecane-3,9-diamine (a cyclic dipeptide of D-ornithine) (Fig. 3).

This is the first report of this newly identified compound from a natural material. One of the antibiotics, β-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.

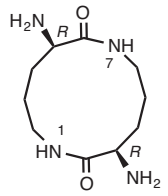

Fig. 3. Structure of Compound **1**.

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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