Toxicity Study on a Marine Snail *Turbo argyrostoma*-III

Occurrence of Candicine

Takeshi YASUMOTO*1 and Mitsuharu ENDO*2

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Previous work on the viscera of a marine snail *Turbo argyrostoma* has revealed the presence of (3-methylthiopropyl) trimethylammonium chloride and an unidentified quaternary amine containing sulfur. In the present study, the occurrence of another amine having similar pharmacological activity was recognized. This third amine differed from the previous two in lacking sulfur and having a phenolic moiety. It was isolated by silicic acid column chromatography and was identified by chemical and spectral analyses as candicine chloride [(p-hydroxyphenethyl) trimethylammonium chloride].

Previously, two toxic amines named toxins A and B were isolated from the viscera of a marine snail *Turbo argyrostoma* collected in Okinawa. Both amines were found to contain sulfur in the molecule and the major compound was identified as (3-methylthiopropyl) trimethylammonium chloride. In the present study, presence of another amine having a similar pharmacological activity was recognized. This third amine was isolated and shown to differ from the previous two amines in lacking sulfur and having a phenolic group. Chemical and spectral analyses on the purified sample indicated that it is candicine chloride [(p-hydroxyphenethyl) trimethylammonium chloride]. The structure was confirmed by comparison with synthetic specimens.

Material and Methods

Specimens of *T. argyrostoma* were collected at the coast of Motobu Peninsula of Okinawa Island in August 1972. Soon after catch, the viscera were cut out, combined, and kept frozen until used.

From 3 kg of the viscera, the acetone soluble fraction was prepared and chromatographed on acidic alumina and on silicic acid columns as previously described for the isolation of toxin A. The final stage of purification was carried out by partition chromatography on a silicic acid column (3.2 × 60 cm) with a solvent consisting of 1-butanol, acetic acid, and water (4:1:2). The eluate was collected in 20 ml portions and was checked by thin layer chromatography. The fractions containing a single component were combined

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*1 Laboratory of Food Science, Faculty of Agriculture, Tohoku University, Sendai (安元 稔・遠藤光幸：東北大学農学部食糧化学科)

*2 Present address: Research Institute, Meiji Milk Products Co., 1-21-3 Sakae-cho, Higashimurayama-shi, Tokyo (遠藤光幸：明治乳業株式会社研究所)
and evaporated. The residue judged as homogeneous by thin layer chromatography was used for color reactions and measurements of UV and mass spectra. The remaining part was converted to the iodide by treating with potassium iodide in ethanol. The precipitated iodide was crystallized twice from ethanol and used for combustion analysis and IR spectrometry.

The UV spectra were run on a Hitachi recording spectrometer, IR spectra on a Japan Spectroscopic Co. model IR-S spectrometer, and mass spectra on a JEOL model MS-06 mass spectrometer.

For thin layer chromatography, the following conditions were adopted: (A) Wakogel B-5 and methanol-pyridine-acetic acid-water (6:9:4:1), (B) Wakogel B-5 and 1-butanol-acetic acid-water (4:1:2), (C) Aluminium oxide G and 1-propanol-28% ammonium hydroxide (9:1). The Dragendorff reagent and potassium ferricyanide-ferric chloride reagent3 were used for detection of the spots.

Synthetic candicine iodide was prepared by treating hordenin with methyl iodide in methanol for 30 min. After evaporation to dryness, the residue was crystallized once from water and twice from ethanol. A part of the synthetic sample was converted to the chloride by treating in methanol with silver oxide followed by acidification with hydrochloric acid.

Results

Candicine was eluted from the silicic acid column in fractions Nos. 50~90. Evaporation of the fractions Nos. 50~69 yielded 33 mg of candicine judged as homogeneous by thin layer chromatography. The residue obtained from subsequent fractions Nos. 70~90 was contaminated by other components. It was rechromatographed similarly and 10 mg of the homogeneous sample was obtained.

The sample thus isolated was indistinguishable from the synthesized specimen of candicine chloride in thin layer chromatography. The Rf values were 0.42, 0.26, and 0.56 in solvents A, B, and C, respectively. The UV spectra of the two samples are identical as shown in Fig. 1 (λmax = 280 mÅ, ε = 3.46).

The mass spectrum of the isolated sample (Fig. 2) is in a good agreement with that of synthetic candicine chloride. A fragment ion at m/e 134 fits CH₃OC₆H₄H₂CH=CH₂ but it does not necessarily mean that CH₃O moiety is originally present. Since the same peak was seen in the spectrum of the synthetic sample, it may arise from methylation of the hydroxyl group during thermal decomposition of the sample. Additional evidences were obtained after conversion of the isolated sample into the iodide, which is less soluble than the chloride and, therefore, is suited for handling in a small quantity. The iodide was obtained as needles. Mp. 229~229.6°C. Anal. Found: C, 42.81; H, 5.78; N, 4.15%.
Calcd. for C_{11}H_{18}INO: C, 43.01; H, 5.91; N, 4.50%. Synthetic candicine iodide was obtained in a yield of 80%. Mp. 229~230°C. Anal. Found: C, 43.03; H, 5.92; N, 4.25%. Chloride, mp. 208~210°C (lit.⁴ mp. 209~210°C). The IR spectra of both isolated and synthesized specimens of candicine iodide are identical as shown in Fig. 3.

When the candicine sample obtained by silicic acid column chromatography was in-
jected intraperitoneally at a dose of 80 mg/kg, the mice developed strong paralysis in limbs and died from respiratory failure within 10 min.

Discussion

Occurrence of candicine in the viscera of Okinawa *T. argyrostoma* was established by the present work. This compound has been known to occur in several terrestrial plants and, in the animal kingdom, in the skin of a few species of leptodactylid frog. Its occurrence in marine organisms, however, seems to have not yet been reported. At present its biogenic origin is still unknown, but its wide distribution in the plant kingdom seems to suggest that it is taken up from the algae by the gastropods.

Symptoms in mice caused by *i. p.* injection of candicine were similar to those caused by toxins A and B but were distinctly different from those induced by ciguatoxin which is also believed to be a product of benthic algae or microorganisms around the coral reef. According to Craig candicine has prominent curariform, stimulating nicotinic, and paralyzing nicotinic actions and the LD$_{50}$ in mice (*i. p. injection, iodide*) is 55 mg/kg. These pharmacological activities may be comparable to those of tetramine which is considered to be a causative agent in poisoning from the ingestion of the salivary gland of some gastropods of the genus *Neptunea*. Although it is unlikely that candicine in *T. argyrostoma* was responsible for the marine snail poisoning occurred in Marcus Island, presence of candicine should be borne in mind when utilizing the gastropod as food.

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