smoke stops evolving. Heat strongly between 900°C and 950°C to constant mass, cool, and weigh as titanium oxide ($TiO_2$).

**Containers and storage** Containers—Well-closed containers.

# Toad Venom

*Bufonis Venenum*

センソ

Toad Venom is the venomous secretion of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider (*Bufonidae*).

When dried, it contains not less than 5.8% of bufo steroid.

**Description** A round disk with slightly dented bottom and protuberant surface, about 8 cm in diameter, about 1.5 cm in thickness, the mass of one disk being about 80 to 90 g; or a round disk with almost flattened surfaces on both sides, about 3 cm in diameter, and about 0.5 cm in thickness, the mass of one disk being about 8 g; externally red-brown to blackish brown, somewhat lustrous, approximately uniform and horny, hard in texture, and difficult to break; fractured surface nearly flat, and edges of broken pieces red-brown and translucent. Odorless; taste, bitter and irritating, followed a little later by a lasting sensation of numbness.

**Identification** **(1)** Warm 0.1 g of pulverized Toad Venom with 5 mL of chloroform under a reflux condenser on a water bath for 10 minutes, filter, and perform the following tests using the filtrate as the sample solution.

(i) To 1 mL of the sample solution add carefully 1 mL of sulfuric acid to make two layers; a vivid yellow color develops at the zone of contact, then changes to red after standing for 15 to 20 minutes, and the chloroform layer acquires a light red color.

(ii) Evaporate 1 mL of the sample solution on a water bath to dryness, dissolve the residue in 25 mL of methanol, and determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry: it exhibits a maximum at about 300 nm.

**(2)** Warm 0.1 g of pulverized Toad Venom with 5 mL of a solution of L-tartaric acid (1 in 100) in a water bath for 10 minutes, and filter. To 1 mL of the filtrate add carefully 1 mL of 4-dimethylaminobenzaldehyde TS, heat for 10 minutes in a water bath, and add 10 mL of water: a blue color develops.

**Total ash** Not more than 5.0%.

**Acid-insoluble ash** Not more than 2.0%.

**Component determination** Weigh accurately about 0.5 g of pulverized Toad Venom, previously dried in a desiccator (silica gel) for 24 hours, add 50 mL of methanol, heat under a reflux condenser on a water bath for 1 hour, cool, and filter. Wash the residue with 30 mL of methanol, and combine the washing and filtrate. To this solution add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g, about 0.02 g and about 0.02 g of bufalin for component determination, cinobufagin for component determination and resibufogenin for component determination, respectively, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 10 $\mu$L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine the ratios, $Q_{TB}$ and $Q_{SB}$, of the peak area of bufalin, $Q_{TC}$ and $Q_{SC}$, of the peak area of cinobufagin, and $Q_{TR}$ and $Q_{SR}$, of the peak area of resibufogenin, respectively, to that of the internal standard in each solution, and designate the total amount as an amount of bufosteroid.

$$\text{Amount (mg) of bufalin} = \text{amount (mg) of bufalin for component determination} \times \frac{Q_{TB}}{Q_{SB}}$$

$$\text{Amount (mg) of cinobufagin} = \text{amount (mg) of cinobufagin for component determination} \times \frac{Q_{TC}}{Q_{SC}}$$

$$\text{Amount (mg) of resibufogenin} = \text{amount (mg) of resibufogenin for component determination} \times \frac{Q_{TR}}{Q_{SR}}$$

*Internal standard solution*—A solution of indometacin in methanol (1 in 4000).

*Operating conditions*—

Detector: An ultraviolet spectrophotometer (wavelength: 300 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 $\mu$m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is 16 to 19 minutes.

Selection of column: Proceed with 10 $\mu$L of the standard solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin, resibufogenin and the internal standard in this order, and clearly dividing each peak.