7 mL of freshly prepared bromine TS, 40 mL of diluted phosphoric acid (1 in 2), and boil until starch iodide paper is no longer colored blue by the evolved gas. Wash down inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume at not less than 200 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.21151 mg of I

Containers and storage Containers—Tight containers.

Titanium Oxide

酸化チタン

TiO_2: 79.87

Titanium Oxide, when dried, contains not less than 98.5% of TiO_2.

Description Titanium Oxide occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It dissolves in hot sulfuric acid and in hydrofluoric acid, and does not dissolve in hydrochloric acid, in nitric acid and in dilute sulfuric acid.

When fused by heating with potassium hydrogen sulfate, with potassium hydroxide, or with potassium carbonate, it changes to soluble salts.

Shake 1 g of Titanium Oxide with 10 mL of water: the mixture is neutral.

Identification Heat 0.5 g of Titanium Oxide with 5 mL of sulfuric acid until white fumes are evolved, cool, add cautiously water to make 100 mL, and filter. To 5 mL of the filtrate add 2 to 3 drops of hydrogen peroxide TS: a yellow-red color develops.

Purity **(1)** Lead—Place 1.0 g of Titanium Oxide in a platinum crucible, add 10.0 g of potassium hydrogen sulfate, heat gently with caution at the beginning, then raise the temperature gradually, and heat strongly with occasional shaking until the contents fuse to yield a clear liquid. Cool, add 30 mL of a solution of diammonium hydrogen citrate (9 in 20) and 50 mL of water, dissolve by heating on a water bath, cool, add water to make 100 mL, and use this solution as the sample stock solution. Take 25 mL of the solution to a separator, add 10 mL of a solution of ammonium sulfate (2 in 5) and 5 drops of thymol blue TS, neutralize with ammonia TS, and add 2.5 mL of ammonia TS. To this solution add exactly 20 mL of a solution of dithizone in *n*-butyl acetate (1 in 500), shake for 10 minutes, and use this *n*-butyl acetate solution as the sample solution. Separately, place 6.0 mL of Standard Lead Solution in a platinum crucible, proceed as directed in the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions: the absorbance of the sample solution is smaller than that of the standard solution (not more than 60 ppm).

Gas: Combustible gas—Acetylene gas or hydrogen gas
Supporting gas—Air
Lamp: Lead hollow-cathode lamp
Wavelength: 283.3 nm

(2) Arsenic—Perform the test using apparatus B with 20 mL of the sample stock solution obtained in (1) as the test solution: the stain is not deeper than the following standard stain.

Standard stain: Proceed in the same manner without Titanium Oxide, transfer 20 mL of the obtained solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(3) Water-soluble substances—Shake thoroughly 4.0 g of Titanium Oxide with 50 mL of water, and allow to stand overnight. Shake thoroughly with 2 mL of ammonium chloride TS, add further 2 mL of ammonium chloride TS if necessary, and allow titanium oxide to settle. Add water to make 200 mL, shake thoroughly, and filter through double filter paper. Discard the first 10 mL of the filtrate, evaporate 100 mL of the clear filtrate on a water bath, and heat strongly at 650°C to constant mass: the mass of the residue is not more than 5.0 mg.

Loss on drying Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.2 g of Titanium Oxide, previously dried, transfer to a crucible, and add 3 g of potassium disulfate. Cover, and heat gently at first, gradually raise the temperature, and then heat the fused contents for 30 minutes. Continue heating for 30 minutes at a higher temperature to make the fused mixture a deep yellow-red, almost clear liquid. Cool, transfer the contents of the crucible to a 250-mL beaker, wash the crucible with a mixture of 75 mL of water and 2.5 mL of sulfuric acid into the beaker, and heat on a water bath until the solution becomes almost clear. Dissolve 2 g of L-tartaric acid in the solution, add 2 to 3 drops of bromothymol blue TS, neutralize with ammonia TS, and acidify with 1 to 2 mL of diluted sulfuric acid (1 in 2). Pass hydrogen sulfide sufficiently through the solution, add 30 mL of ammonia TS, again saturate the solution with hydrogen sulfide, allow to stand for 10 minutes, and filter. Wash the precipitate on the filter paper with ten 25-mL portions of a solution of ammonium L-tartrale (1 in 100), containing 2.5 mL of ammonium sulfide TS. When the precipitate is filtered and washed, prevent iron (II) sulfide from oxidation by filling the solution on the filter paper. Combine the filtrate and the washings, add 40 mL of diluted sulfuric acid (1 in 2), and boil to expel hydrogen sulfide. Cool, and dilute with water to make 400 mL. Add gradually 40 mL of cupferron TS to the solution with stirring, and allow to stand. After sedimentation of a yellow precipitate, add again cupferron TS until a white precipitate is produced. Filter by slight suction using quantitative filter paper, wash with twenty portions of diluted hydrochloric acid (1 in 10), and remove water by stronger suction at the last washing. Dry the precipitate together with the filter paper at 70°C, transfer to a tared crucible, and heat very gently at first, and raise the temperature gradually after

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 μL 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 μm 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 μL 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.