shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

(2) To 1 mL of the aqueous layer obtained in (1) after extraction with diethyl ether, add 2 drops of iron (III) chloride TS: a bluish-black color develops. Allow to stand: a bluish-black precipitate is formed (tannic acid).

(3) To 5 g of Compound Scopolia Extract and Tannic Acid Ointment add 10 mL of hot water, heat on a water bath for 10 minutes with occasional stirring, and then cool in ice. Remove the coagulation on the solution with a glass rod, filter, and boil 5 mL of the filtrate with 5 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue (ichthammol).

(4) To 6 g of Compound Scopolia and Tannic Acid Ointment add 10 mL of water, stir well while warming on a water bath, cool in ice, and filter. Make the filtrate alkaline with ammonia TS, add 10 mL of diethyl ether, and shake vigorously. Separate the diethyl ether layer, add 3 g of anhydrous sodium sulfate to the diethyl ether solution. Shake, and filter when the diethyl ether layer becomes clear. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 0.020 g of atropine sulfate for thin-layer chromatography and 0.010 g of scopolamine hydrobromide in 10 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution show the same in color tone and *Rf* value with each yellow-red spot from the standard solutions, respectively.

Containers and storage Containers—Tight containers.

Scopolia Rhizome

Scopoliae Rhizoma

ロートコン

Scopolia Rhizome is the rhizome and root of *Scopolia japonica* Maximowicz, *Scopolia carniolica* Jacquin or *Scopolia parviflora* Nakai (*Solanaceae*).

When dried, it contains not less than 0.29% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

Description Chiefly irregularly branched, slightly curved rhizome, about 15 cm in length, about 3 cm in diameter, occasionally longitudinally cut; externally grayish brown, with wrinkles; constrictions make the rhizome appear nodular; rarely, stem base at one end; stem scars at upper side of each node; roots or root scars on both sides and lower surface of rhizome; fractured surface granular, grayish white to light brown in color, with lighter colored cortex. Odor characteristic; taste sweet, later slightly bitter.

Under a microscope, xylem reveals groups of vessels arranged stepwise, and accompanied with xylem sieve tubes in medullary rays; parenchyma cells contain starch grains, and sometimes sand crystals of calcium oxalate.

Identification **(1)** To 1 g of pulverized Scopolia Rhizome add 10 mL of diethyl ether and 0.5 mL of ammonia TS, shake for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, transfer the filtrate and the washing to a separator, add 20 mL of diluted sulfuric acid (1 in 50), shake well, and drain off the acid extract into another separator. Render the solution slightly alkaline with ammonia TS, add 10 mL of diethyl ether, shake well, transfer the diethyl ether layer to a porcelain dish, and evaporate the diethyl ether on a water bath. To the residue add 5 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Cool, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple to purple color develops.

(2) Place 2.0 g of pulverized Scopolia Rhizome in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate Reference Standard and 1 mg of Scopolamine Hydrobromide Reference Standard in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution and each yellow-red spot from the standard solutions show the same color tone and the same *Rf* value.

(3) To 3 g of pulverized Scopolia Rhizome add 10 mL of chloroform, shake thoroughly, and filter. To 5 mL of the filtrate add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

Total ash Not more than 7.0%.

Assay Weigh accurately about 0.7 g of pulverized Scopolia Rhizome, previously dried at 60°C for 8 hours, in a glass-stoppered, centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.025 g of Atropine Sulfate Reference Standard (determine the loss

on drying before use), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 0.025 g of Scopolamine Hydrobromide Reference Standard (determine the loss on drying before use), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of standard stock solution A and 1 mL of standard stock solution B, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, 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_{TA} and Q_{SA}, of the peak area of hyoscyamine (atropine), and the ratios, Q_{TS} and Q_{SS}, of the peak area of scopolamine to that of the internal standard in each solution, calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

Amount (mg) of hyoscyamine ($C_{17}H_{23}NO_3$)
= amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis
$\times \frac{Q_{TA}}{Q_{SA}} \times \frac{1}{5} \times 0.855$

Amount (mg) of scopolamine ($C_{17}H_{21}NO_4$)
= amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis
$\times \frac{Q_{TS}}{Q_{SS}} \times \frac{1}{25} \times 0.789$

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadesilcylanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to a pH of 3.5, and add water to make 1000 mL. To 9 parts of this solution add 1 part of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and the resolution between the peaks of atropine and the internal standard being not less than 4.

Compound Scopolia Extract and Tannic Acid Suppositories

複方ロートエキス・タンニン坐剤

Method of preparation

Scopolia Extract	0.2 g
Tannic Acid	0.3 g
Ichthammol	2.0 g
Ethyl Aminobenzoate	1 g
Cacao Butter or a suitable base	a sufficient quantity

Prepare 10 suppositories as directed under Suppositories with the above ingredients.

Description Compound Scopolia Extract and Tannic Acid Suppositories are blackish brown suppositories, having a characteristic odor.

Identification **(1)** Shake 2 Compound Scopolia Extract and Tannic Acid Suppositories with 20 mL of diethyl ether for 10 minutes to dissolve the base of suppositories. Shake thoroughly the mixture with 15 mL of water, separate the water layer, and filter. To the filtrate add 10 mL of chloroform, shake well, and separate the chloroform layer. Take 5 mL of the chloroform solution, add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

(2) To 1 mL of the aqueous layer obtained in (1) after extraction with diethyl ether, add 2 drops of iron (III) chloride TS: a bluish-black color develops. Allow to stand: a bluish-black precipitate is formed (tannic acid).

(3) To 2 Compound Scopolia Extract and Tannic Acid Suppositories add 10 mL of hot water, heat on a water bath for 10 minutes with occasional stirring, and cool in ice. Remove the coagulation on the solution with a glass rod, and filter. Boil 5 mL of the filtrate with 5 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue (ichthammol).

(4) To 1 suppository of Compound Scopolia Extract and Tannic Acid Suppositories add 40 mL of ethanol (95). Warm for 20 minutes on a water bath with stirring. Cool in ice, centrifuge, and filter the supernatant liquid. To 1 mL of the filtrate add 4 mL of ethanol (95), and use this solution as the sample solution. Dissolve 0.025 g of ethyl aminobenzoate in 50 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μL each of the sample solution and the standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *Rf* value.

Containers and storage Containers—Well-closed containers.