and not more than 1.05% of hyoscyamine ($C_{17}H_{23}NO_3$: 389.37).

Method of preparation To 1000 g of a coarse powder of Belladonna Root add 4000 mL of 35 vol% ethanol, and digest for 3 days. Press the mixture, add 2000 mL of 35 vol% ethanol to the residue, and digest again for 2 days. Combine all the extracts, and allow to stand for 2 days. Filter, and prepare the viscous extract as directed under Extracts. May be prepared with an appropriate quantity of Ethanol and Purified Water.

Description Belladonna Extract has a dark brown color, a characteristic odor and a bitter taste.

Identification Mix 0.5 g of Belladonna Extract with 30 mL of ammonia TS in a flask, transfer the mixture to a separator, then add 40 mL of ethyl acetate, and shake the mixture. 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. Proceed as directed in the Identification under Belladonna Root.

Assay Weigh accurately about 0.4 g of Belladonna Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine 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 exactly 25 mL. Proceed as directed under Belladonna Root.

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

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

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Belladonna Root

Belladonnae Radix

ベラドンナコン

Belladonna Root is the root of *Atropa belladonna* Linné (*Solanaceae*).

Belladonna Root, when dried, contains not less than 0.4% of hyoscyamine ($C_{17}H_{23}NO_3$: 289.37).

Description Cylindrical root, usually 10 - 30 cm in length, 0.5 - 4 cm in diameter; often cut crosswise or lengthwise; externally grayish brown to grayish yellow-brown, with longitudinal wrinkles; periderm often removed; fractured surface is light yellow to light yellow-brown in color and is powdery. Almost odorless; taste, bitter.

Identification Place 2.0 g of pulverized Belladonna Root 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 in 1 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 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: the principal spot from the sample solution is the same in color tone and *Rf* value with a yellow-red spot from the standard solution.

Purity **(1)** Stem and crown—The amount of stems and crowns contained in Belladonna Root does not exceed 10.0%.

(2) Foreign matter—The amount of foreign matter other than stems and crowns contained in Belladonna Root does not exceed 2.0%.

Total ash Not more than 6.0%.

Acid-insoluble ash Not more than 4.0%.

Assay Weigh accurately about 0.7 g of pulverized Belladonna Root, previously dried at 60°C for 8 hours, place 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 exactly 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. Pipet 5 mL of standard stock solution, 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_T and Q_S, of the peak area of hyoscyamine (atropine), to that of the internal standard in each solution.

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

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 about 4 mm in inside diameter and about 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, and mix this solution with acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of atropine is about 14 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and determine the resolution. Use a column giving elution of atropine and the internal standard in this order with the resolution between these peaks being not less than 4.

Bentonite

ベントナイト

Bentonite is a natural, colloidal, hydrated aluminum silicate.

Description Bentonite occurs as a very fine, white to light yellow-brown powder. It is odorless. It has a slightly earthy taste.

It is practically insoluble in water and in diethyl ether.

It swells in water.

Identification **(1)** Add 0.5 g of Bentonite to 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. Cool, add 20 mL of water, and filter. To 5 mL of the filtrate add 3 mL of ammonia TS: a white, gelatinous precipitate is produced, which turns red on the addition of 5 drops of alizarin red S TS.

(2) Wash the residue obtained in (1) with water, add 2 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash again with water: the residue is blue in color.

pH To 1.0 g of Bentonite add 50 mL of water, and shake: the pH of the suspension is between 9.0 and 10.5.

Purity **(1)** Heavy metals—To 1.5 g of Bentonite add 80 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes with thorough stirring. Cool, centrifuge, collect the supernatant liquid, wash the residue with two 10-mL portions of water, and centrifuge each. Combine the supernatant liquid and the washings, and add dropwise ammonia solution (28). When a precipitate is produced, add dropwise dilute hydrochloric acid with vigorous stirring, and dissolve. To the solution add 0.45 g of hydroxylammonium chloride, and heat. Cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Pipet 50 mL of the solution, and perform the test using this solution as the test solution. Prepare the control solution as follows: mix 2.5 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 50 ppm).

(2) Arsenic—To 1.0 g of Bentonite add 5 mL of dilute hydrochloric acid, and gently heat to boil while stirring well. Cool immediately, and centrifuge. To the residue add 5 mL of dilute hydrochloric acid, shake well, and centrifuge. To the residue add 10 mL of water, and perform the same operations. Combine all the extracts, and heat on a water bath to concentrate to 5 mL. Perform the test with this solution as the test solution using Apparatus B (not more than 2 ppm).

(3) Foreign matter—Place 2.0 g of Bentonite in a mortar, add 20 mL of water to swell, disperse evenly with a pestle, and dilute with water to 100 mL. Pour the suspension through a No. 200 (74 μm) sieve, and wash the sieve thoroughly with water. No grit is felt when the fingers are rubbed over the wire mesh of the sieve.

Loss on drying 5.0 - 10.0% (2 g, 105°C, 2 hours).

Gel formation Mix 6.0 g of Bentonite with 0.30 g of magnesium oxide. Add the mixture, in several portions, to 200 mL of water contained in a glass-stoppered 500-mL cylinder. Agitate for 1 hour, transfer 100 mL of the suspension to a 100-mL graduated cylinder, and allow to stand for 24 hours: not more than 2 mL of supernatant appears on the surface.

Swelling power To 100 mL of water in a glass-stoppered 100-mL cylinder add 2.0 g of Bentonite in ten portions, allowing each portion to settle before adding the next, and allow to stand for 24 hours: the apparent volume of the sediment at the bottom is not less than 20 mL.

Containers and storage Containers—Well-closed containers.

Benzoin

Benzoinum

アンソッコウ

Benzoin is the resin obtained from *Styrax benzoin* Dryander or other species of the same genus (*Styracaceae*).

Description Benzoin occurs as grayish brown to dark red-brown blocks varying in size; the fractured surface exhibiting whitish to light yellow-red grains in the matrix; hard and brittle at ordinary temperature but softened by heat. Odor, characteristic and aromatic; taste, slightly pungent and acrid.

Identification **(1)** Heat a fragment of Benzoin in a test tube: it evolves an irritating vapor, and a crystalline sublimate is produced.

(2) Digest 0.5 g of Benzoin with 10 mL of diethyl ether, decant 1 mL of the diethyl ether into a porcelain dish, and