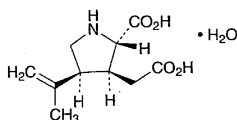


Description Josamycin Propionate occurs as a white to light yellowish white crystalline powder.

It is freely soluble in methanol and in ethanol (95), soluble in diethyl ether, and practically insoluble in water.

Kainic Acid

カイニン酸



$C_{10}H_{15}NO_4 \cdot H_2O$: 231.25
(2*S*,3*S*,4*S*)-3-(Carboxymethyl)-4-isopropenylpyrrolidine-2-carboxylic acid monohydrate [487-79-6, anhydride]

Kainic Acid, when dried, contains not less than 99.0% of $C_{10}H_{15}NO_4$: 213.23.

Description Kainic Acid occurs as white crystals or crystalline powder. It is odorless, and has an acid taste.

It is sparingly soluble in water and in warm water, very slightly soluble in acetic acid (100) and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

The pH of its solution (1 in 100) is between 2.8 and 3.5. Melting point: about 252°C (with decomposition).

Identification (1) To 5 mL of a solution of Kainic Acid (1 in 5000) add 1 mL of ninhydrin TS, and warm in a water bath at a temperature between 60°C and 70°C for 5 minutes: a yellow color is produced.

(2) Dissolve 0.05 g of Kainic Acid in 5 mL of acetic acid (100), and add 0.5 mL of bromine TS: the color of bromine disappears immediately.

Optical rotation $[\alpha]_D^{20}$: -13 - -17° (0.5 g, water, 50 mL, 200 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Kainic Acid in 10 mL of water: the solution is clear and colorless.

(2) Chloride—Take 0.5 g of Kainic Acid in a platinum crucible, dissolve in 5 mL of sodium carbonate TS, and evaporate on a water bath to dryness. Heat the crucible slowly at first, and then ignite until the sample is almost incinerated. After cooling, add 12 mL of dilute nitric acid to the residue, dissolve by warming, and filter. Wash the residue with 15 mL of water, combine the washings and the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution.

Control solution: Add 5 mL of sodium carbonate TS to 0.30 mL of 0.01 mol/L hydrochloric acid VS, and proceed as directed above (not more than 0.021%).

(3) Sulfate—Dissolve 0.5 g of Kainic Acid in 40 mL of water by warming. Cool, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium—Take 0.25 g of Kainic Acid, and perform the test. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals—Proceed with 1.0 g of Kainic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic—Dissolve 1.0 g of Kainic Acid in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution using Apparatus B (not more than 2 ppm).

(7) Amino acid and other imino acid—Dissolve 0.10 g of Kainic Acid in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of this solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under the Thin-layer Chromatography with these solutions. Spot 10 μ L each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant liquid of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying 6.5 - 8.5% (1 g, 105°C, 4 hours).

Residue on ignition Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Kainic Acid, previously dried, and dissolve in 50 mL of warm water, cool and titrate with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.323 mg of $C_{10}H_{15}NO_4$

Containers and storage Containers—Tight containers.

Kallidinogenase

カリジノゲナーゼ

[9001-01-8]

Kallidinogenase is an enzyme obtained from healthy porcine pancreas, and has kinin-releasing activity based on cleavage of kininogen. It contains not less than 25 Kallidinogenase Units per mg. Usually, it is diluted with Lactose or the like.

Kallidinogenase contains not less than 90% and not more than 110% of the labeled Units.

Description Kallidinogenase occurs as a white to light brown powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Kallidinogenase (1 in 300) is between 5.5 and 7.5.

Identification (1) Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 10 Kallidinogenase Units per mL. Pipet 5 mL of this solution, and add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each test tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 1 and 2. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 3 and 4. Then, pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.5 mL of the sample solution 1 warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, and start simultaneously a chronograph. Perform the test at $30.0 \pm 0.5^\circ\text{C}$ as directed under the Ultraviolet-visible Spectrophotometry using water as the blank, and determine the absorbances at 405 nm, A_{1-2} and A_{1-6} , of this solution, after having allowed it to stand for exactly 2 and 6 minutes. Perform the same test with the sample solutions 2, 3 and 4, and determine the absorbances, A_{2-2} , A_{2-6} , A_{3-2} , A_{3-6} , A_{4-2} and A_{4-6} , of these solutions. Calculate I by using the following equation: the value of I does not exceed 0.2.

$$I = \frac{(A_{1-6} - A_{1-2}) - (A_{3-6} - A_{3-2})}{(A_{2-6} - A_{2-2}) - (A_{4-6} - A_{4-2})}$$

(2) Pipet 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.1 mL of the sample solution obtained in the Assay, and start simultaneously a chronograph. Perform the test at $30.0 \pm 0.5^\circ\text{C}$ as directed under the Ultraviolet-visible Spectrophotometry, and determine the change of the absorbance at 253 nm for 4 to 6 minutes. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Add exactly 0.1 mL of this solution to exactly 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, and use this solution as the blank. If the rate of change in the absorbance remains constant, determine the change of absorbance per 1 minute, A , and calculate R by using the following equation: the value of R is between 0.12 and 0.16.

$$R = \frac{A}{0.0383} \times \frac{1}{a \times b}$$

a : Amount (mg) of Kallidinogenase in 1 mL of the sample solution.

b : Amount (Unit) of kallidinogenase in 1 mg of Kallidinogenase obtained in the Assay.

Specific activity Perform the test with Kallidinogenase as directed under the Nitrogen Determination to determine the nitrogen content, convert 1mg of nitrogen (N:14.007) into 6.25 mg of protein, and calculate the specific activity using

the amount (Units) of Kallidinogenase obtained in the Assay: it is not less than 100 Kallidinogenase Units per 1mg of protein.

Purity (1) Fat—To 1.0 g of Kallidinogenase add 20 mL of diethyl ether, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1 mg.

(2) Kininase—

(i) Bradykinin solution: Weigh an appropriate amount of bradykinin, and dissolve in gelatin-phosphate buffer solution, pH 7.4 to prepare a solution containing $0.200 \mu\text{g}$ of bradykinin per mL.

(ii) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase according to the labeled unit, dissolve in gelatin-phosphate buffer solution, pH 7.4 to make a solution containing 1 unit of kallidinogenase per mL.

(iii) Sample solution: Pipet 0.5 mL of bradykinin solution, warm at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and mix immediately. After allow this solution to stand at $30 \pm 0.5^\circ\text{C}$ for exactly 150 seconds, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and mix. Pipet 0.1 mL of this solution, add exactly 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution, and mix. Pipet 0.2 mL of this solution, add exactly 0.6 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iv) Control solution: Proceed with 0.5 mL of gelatin-phosphate buffer solution, pH 7.4 as described in (iii), and use the solution so obtained as the control solution.

(v) Procedure: Add 0.1 mL of anti-bradykinin antibody TS to anti-rabbit antibody-coated wells of a 96-well microplate, shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Remove the anti-bradykinin antibody TS, add 0.3 mL of phosphate buffer solution for microplate washing to the wells, then remove. Repeat this procedure 3 times, take off the washings thoroughly, then add $100 \mu\text{L}$ each of the sample solution and the control solution, and $50 \mu\text{L}$ of gelatin-phosphate buffer solution, pH 7.0, shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Then add $50 \mu\text{L}$ of peroxidase-labeled bradykinin TS, shake, and allow to stand in a cold place for a night.

Take off the solution, add 0.3 mL of phosphate buffer solution for microplate washing, and remove. Repeat this procedure more 4 times, take off the washings thoroughly, add $100 \mu\text{L}$ of substrate solution for peroxidase determination, and allow to stand at a constant temperature of about 25°C for exactly 30 minutes while protecting from light. Then add $100 \mu\text{L}$ of diluted sulfuric acid (23 in 500), shake, and determine the absorbance at 490–492 nm as directed under the Ultraviolet-visible Spectrophotometry.

Separately, dissolve a suitable amount of bradykinin in gelatin-phosphate buffer solution, pH 7.0 to make solutions containing exactly 100 ng, 25 ng, 6.25 ng, 1.56 ng, 0.39 ng

and 0.098 ng of bradykinin per mL, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3), the standard solution (4), the standard solution (5) and the standard solution (6), respectively. Use 1 mL of gelatin-phosphate buffer solution, pH 7.0 as the standard solution (7). To each of the well add 50 μ L each of the standard solutions and 100 μ L of trichloroacetic acid-gelatin-tris buffer solution, and proceed in the same manner as for the sample solution and for the control solution.

Prepare the standard curve from the amounts of bradykinin in the standard solutions and their absorbances, and determine the amount of bradykinin, B_T (pg) and B_S (pg), of the sample solution and the control solution.

The absorbance is usually determined by using a spectrophotometer for microplate. Since the wells are used as the cell for absorbance determination, take care for dirt and scratch of the well. Light pass length of the well is changeable by the amount of the liquid, exact addition of the liquid is necessary.

(vi) Judgment: The value R calculated by the following equation is not less than 0.8.

$$R = \frac{B_T}{B_S}$$

(3) Trypsin-like substances—Pipet 4 mL of the sample stock solution prepared for the Assay, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.5 mL of the sample solution, warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and start simultaneously a chronograph. Perform the test at $30 \pm 0.5^\circ\text{C}$ as directed under the Ultraviolet-visible Spectrophotometry using water as the blank, and determine the absorbances at 405 nm, A_2 and A_6 , of this solution after having allowed it to stand for exactly 2 and 6 minutes. Separately, pipet 4 mL of the sample stock solution prepared for the Assay, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the control solution. Perform the same test with the control solution, and determine the absorbances, A_2' and A_6' . Calculate T by using the following equation: the value of T does not exceed 0.05.

$$T = \frac{(A_6' - A_2') - (A_6 - A_2)}{(A_6' - A_2')}$$

(4) Protease—Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 1 Kallidinogenase Unit per mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, place in a test tube, and allow to stand at $35 \pm 0.5^\circ\text{C}$ for 5 minutes. Then, pipet 5 mL of substrate TS for kallidinogenase assay (3), previously warmed to $35 \pm 0.5^\circ\text{C}$, add quickly to the sample solution in the test tube, and allow to stand at $35 \pm 0.5^\circ\text{C}$ for exactly 20 minutes. Then add exactly 5 mL of trichloroacetic acid TS, shake well, allow to stand at room temperature for 1 hour, and filter through a membrane filter (5 μ m in pore size). Discard the first 3 mL of the filtrate, and determine the absorbance, A , of the subsequent filtrate at 280 nm within 2 hours as

directed under the Ultraviolet-visible Spectrophotometry, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS, shake well, and add exactly 5 mL of the substrate TS for kallidinogenase assay (3). Proceed in the same manner as described for the sample solution, and determine the absorbance, A_0 , of this solution. Calculate the value of $(A - A_0)$: it is not more than 0.2.

Loss on drying Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition Not more than 3.0% (0.5g, 650–750°C).

Kinin-releasing activity

(i) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase, according to the labeled unit, dissolve in 0.02 mol/L phosphate buffer solution, pH 8.0 to make a solution containing 0.1 unit of kallidinogenase per mL. Perform this procedure by using glassware.

(ii) Sample solution: Pipet 0.5 mL of kininogen TS, warm at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and mix immediately. After allow this solution to stand at $30 \pm 0.5^\circ\text{C}$ for exactly 2 minutes, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. Pipet 0.1 mL of this solution, add exactly 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iii) Procedure: Perform the test with the sample solution as directed in the Purity (2), and determine the amount, B (pg), of kinin per well. The kinin-releasing activity per 1 unit of Kallidinogenase calculated by the following equation is not less than 500 ng bradykinin equivalent/min/unit.

Kinin-releasing activity (ng bradykinin equivalent/min/unit) per 1 unit of Kallidinogenase = $B \times 4.8$

Assay Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 10 Kallidinogenase Units per mL, and use this solution as the sample stock solution. Pipet 4 mL of the sample stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.5 mL of the sample solution, warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and start simultaneously a chronograph. Perform the test at $30 \pm 0.5^\circ\text{C}$ as directed under the Ultraviolet-visible Spectrophotometry using water as the blank, and determine the absorbances at 405 nm, A_{T2} and A_{T6} , of this solution after having allowed it to stand for exactly 2 and 6 minutes. Separately, dissolve the contents of one ampoule of Kallidinogenase Reference Standard in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL of this solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this

solution as the standard solution. Take exactly 0.5 mL of the standard solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, A_{S2} and A_{S6} , of the solution after having allowed it to stand for exactly 2 and 6 minutes. Separately, take exactly 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 0.5 mL of this solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, A_{02} and A_{06} , of the solution after having allowed it to stand for exactly 2 and 6 minutes.

$$\begin{aligned} & \text{Units per 1 mg of Kallidinogenase} \\ &= \frac{(A_{T6} - A_{T2}) - (A_{06} - A_{02})}{(A_{S6} - A_{S2}) - (A_{06} - A_{02})} \times \frac{a}{10} \times \frac{1}{b} \end{aligned}$$

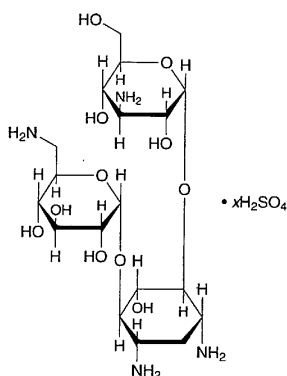
a: Amount (Units) of Kallidinogenase Reference Standard sampled.

b: Amount (mg) of Kallidinogenase in 1 mL of the sample stock solution.

Containers and storage Containers—Tight containers.

Kanamycin Sulfate

硫酸カナマイシン



$C_{18}H_{36}N_4O_{11} \cdot xH_2SO_4$
O-3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-*O*-[6-amino-6-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)]-2-deoxy-D-streptamine sulfate [133-92-6]

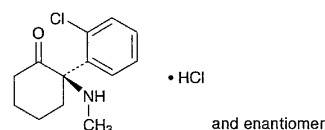
Kanamycin Sulfate conforms to the requirements of Kanamycin Sulfate in the Requirements for Antibiotic Products of Japan.

Description Kanamycin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Ketamine Hydrochloride

塩酸ケタミン



$C_{13}H_{16}ClNO \cdot HCl$: 274.19
(RS)-2-(2-Chlorophenyl)-2-methylaminocyclohexanone monohydrochloride [1867-66-9]

Ketamine Hydrochloride, when dried, contains not less than 99.0% of $C_{13}H_{16}ClNO \cdot HCl$.

Description Ketamine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in water and in methanol, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

A solution of Ketamine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 258°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ketamine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 3000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketamine Hydrochloride, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ketamine Hydrochloride (1 in 10) responds to the Qualitative Tests (2) for chloride.

Absorbance $E_{1\text{cm}}^{1\%}$ (269 nm): 22.0 – 24.5 (after drying, 0.03 g, 0.1 mol/L hydrochloric acid TS, 100 mL).

pH Dissolve 1.0 g of Ketamine Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ketamine Hydrochloride in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals—Proceed with 1.0 g of Ketamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Prepare the test solution with 1.0 g of Ketamine Hydrochloride, according to Method 1, and perform the test using Apparatus B (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Ketamine Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography.