proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

(3) Protamine Sulfate Injection responds to the Qualitative Tests for sulfate.

pH 5.0 - 7.0

Purity Nitrogen—Transfer an exactly measured volume of Protamine Sulfate Injection, equivalent to about 0.010 g of Protamine Sulfate according to the labeled amount, to a Kjeldahl flask, and evaporate on a water bath with the aid of a current of air to dryness. Perform the test as directed under the Nitrogen Determination: 0.225 to 0.255 mg of nitrogen (N: 14.01) is found for each mg of the labeled amount of Protamine Sulfate.

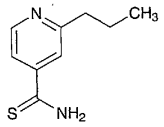
Potency as antiheparin Proceed as directed in the Potency as antiheparin under Protamine Sulfate, but use the following sample solution.

Sample solution: Dilute an exactly measured volume of Protamine Sulfate Injection, equivalent to 20.0 mg of Protamine Sulfate according to the labeled amount, with isotonic sodium chloride solution to make exactly 20 mL.

Containers and storage Containers—Hermetic containers.

Prothionamide

プロチオナミド



$C_9H_{12}N_2S$: 180.27
2-Propylpyridine-4-carbothioamide [*14222-60-7*]

Prothionamide, when dried, contains not less than 98.0% of $C_9H_{12}N_2S$.

Description Prothionamide occurs as yellow crystals or crystalline powder. It has a slight, characteristic odor.

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

It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

Identification **(1)** Mix 0.05 g of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 0.01 g of this mixture to a test tube, and heat for several seconds over a small flame until the mixture is fused. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange-red color develops.

(2) Place 0.5 g of Prothionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and mix well. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt between 198°C and 203°C (with decomposition).

Melting point 142 - 145°C

Purity **(1)** Clarity and color of solution—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear, and shows a yellow color.

(2) Acid—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water bath with agitation, and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature, and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals—Proceed with 1.0 g of Prothionamide 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).

(4) Arsenic—Prepare the test solution with 0.6 g of Prothionamide according to Method 3, and perform the test using Apparatus B. To the test solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of strong hydrogen peroxide, and ignite to burn (not more than 3.3 ppm).

Loss on drying Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.3 g of Prothionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination.

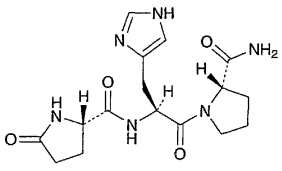
Each mL of 0.1 mol/L perchloric acid VS
= 18.027 mg of $C_9H_{12}N_2S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Protirelin

プロチレリン



$C_{16}H_{22}N_6O_4$: 362.38
5-Oxo-L-prolyl-L-histidyl-L-prolinamide [*24305-27-9*]

Protirelin contains not less than 98.5% of $C_{16}H_{22}N_6O_4$, calculated on the dehydrated basis.

Description Protirelin occurs as a white powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100).

It is hygroscopic.

Identification (1) Take 0.01 g of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube, and heat carefully at 110°C for 5 hours. After cooling, open the seal, transfer the contents into a beaker, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 0.08 g of L-glutamic acid, 0.12 g of L-histidine hydrochloride monohydrate and 0.06 g of L-proline in 20 mL of water, 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 for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the three spots obtained from the sample solution show the same color and the same *Rf* value as each corresponding spots obtained from the standard solution.

(2) Determine the infrared absorption spectrum of Protirelin, 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.

Optical rotation $[\alpha]_D^{20}$: −66.0 - −69.0° (0.1 g calculated on the dehydrated basis, water, 20 mL, 100 mm).

pH Dissolve 0.20 g of Protirelin in 10 mL of water: the pH of this solution is between 7.5 and 8.5.

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

(2) Heavy metals—Proceed with 1.0 g of Protirelin 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).

(3) Other peptides and free amino acids—Dissolve 0.20 g of Protirelin in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water 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. Spot 5 μL each of the sample solution and the standard solution on a plate (1) of silica gel for thin-layer chromatography, and spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of 1-butanol, water, pyridine and acetic acid (100) (4:2:1:1) to a distance of about 12 cm, and dry the plates at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plates. Successively spray evenly a solution of sodium carbonate decahydrate (1 in 10) on it: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and heat at 80°C for 5 minutes: no colored spot appears.

Water Not more than 5.0% (0.1 g, direct titration).

Residue on ignition Not more than 0.3% (0.2 g).

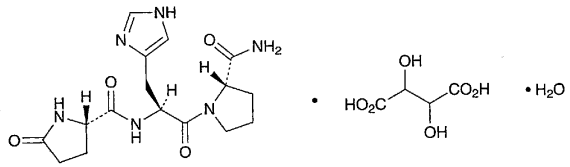
Assay Weigh accurately about 0.07 g of Protirelin dissolve in 50 mL of acetic acid (100), and titrate with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 7.248 mg of $C_{16}H_{22}N_6O_4$

Containers and storage Containers—Tight containers.

Protirelin Tartrate

酒石酸プロチレリン



$C_{16}H_{22}N_6O_4.C_4H_6O_6.H_2O$: 530.49
5-Oxo-L-prolyl-L-histidyl-L-prolinamide monotartrate monohydrate [*24305-27-9*, Protirelin]

Protirelin Tartrate, calculated on the anhydrous basis, contains not less than 98.5% of $C_{16}H_{22}N_6O_4.C_4H_6O_6$: 512.48.

Description Protirelin Tartrate occurs as white to pale yellowish white crystals or crystalline powder.

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

Melting point: about 187°C (with decomposition).

Identification (1) To 1 mL of a solution of Protirelin Tartrate (1 in 1000) add 2 mL of a solution of *p*-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color develops.

(2) Dissolve 0.03 g of Protirelin Tartrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

(3) To 0.20 g of Protirelin Tartrate add 5.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 0.022 g of *l*-glutamic acid, 0.032 g of *l*-histidine hydrochloride (monohydrate) and 0.017 g of *l*-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 2 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry at 80°C for 5 minutes: the three spots ob-