Identification (1) Dissolve 0.01 g of Carbidopa in 250 mL of a solution of hydrochloric acid in methanol (9 in 1000). Determine the absorption spectrum of the solution as directed under the Ultraviolet-visible Spectrophotometry at the wavelengths between 240 nm and 300 nm, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carbidopa Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carbidopa 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 $[\alpha]_D^{20}$: $-21.0 - -23.5°$ (1 g, aluminum (III) chloride TS, 100 mL, 100 mm).

Purity (1) Heavy metals—Proceed with 2.0 g of Carbidopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.050 g of Carbidopa in 70 mL of the mobile phase, by warming and using ultrasonication, if necessary. After cooling, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of all peaks other than the peak of carbidopa from the sample solution is not larger than the peak area of carbidopa from the standard solution.

Operating conditions—

Detector, column, temperature of column, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of carbidopa from 20 μL of the standard solution is about 10% of the full scale.

Time span of measurement: About 3 times as long as the retention time of carbidopa.

Loss on drying 6.9 - 7.9% (1 g, in vacuum not exceeding 0.67 kPa, 100°C, 6 hours).

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

Assay Weigh accurately about 0.05 g each of Carbidopa and Carbidopa Reference Standard (determined separately the loss on drying in the same manner as for Carbidopa), and dissolve each in 70 mL of the mobile phase, by warming and using ultrasonication, if necessary. After cooling, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the peak areas, A_T and A_S, of carbidopa in each solution.

Amount (mg) of $C_{10}H_{14}N_2O_4.H_2O$
= amount (mg) of Carbidopa Reference Standard, calculated on the dried basis

$$\times \frac{A_T}{A_S} \times 1.080$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 950 mL of 0.05 mol/L sodium dihydrogenphosphate TS add 50 mL of ethanol (95), and adjust the pH to 2.7 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of carbidopa is about 6 minutes.

Selection of column: Dissolve 0.05 g each of Carbidopa and methyldopa in 100 mL of the mobile phase. Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of methyldopa and carbidopa in this order with the resolution between these peaks being not less than 0.9.

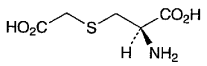
System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of carbidopa is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

L-Carbocisteine

L-カルボシステイン



$C_5H_9NO_4S$: 179.19

(2*R*)-2-Amino-3-carboxymethylsulfanylpropanoic acid

[*638-23-3*]

L-Carbocisteine, when dried, contains not less than 98.5% of $C_5H_9NO_4S$.

Description L-Carbocisteine occurs as a white crystalline powder. It is odorless, and has a slightly acid taste.

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

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

Melting point: about 186°C (with decomposition).

Identification (1) To 0.2 g of L-Carbocisteine add 1 mL of lead acetate TS and 3 mL of water, shake, add 0.2 g of sodium hydroxide, and heat over a flame for 1 minute: a dark brown to black precipitate is formed.

(2) Determine the infrared absorption spectrum of L-Carbocisteine as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and com-

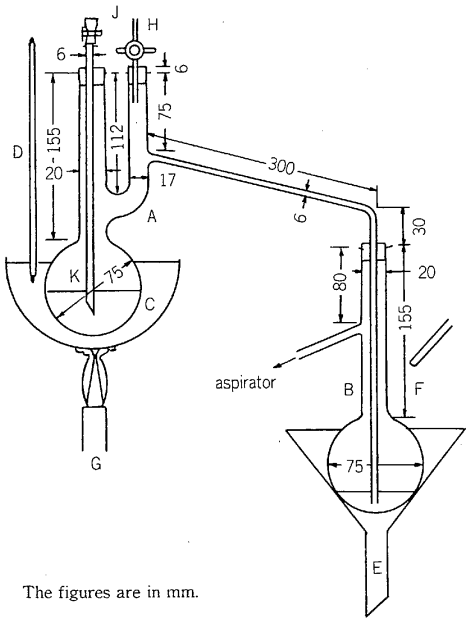
pare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation $[\alpha]_D^{20}$: −33.5 – −36.5° Weigh accurately about 5 g of L-Carbocisteine, previously dried, dissolve in 20 mL of water and a suitable amount of a solution of sodium hydroxide (13 in 100), and adjust the pH with 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS to 6.0, and add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell.

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of L-Carbocisteine in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride—Dissolve 0.20 g of L-Carbocisteine in 10 mL of water and 20 mL of nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(3) Ammonium—(i) Apparatus: Use the apparatus illustrated in the figure. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.



The figures are in mm.

A: Vacuum distilling flask (200 mL)
B: Receiver (200-mL flask)
C: Water bath
D: Thermometer
E: Funnel
F: Cooling water
G: Gas burner
H: Glass cock
J: Rubber tube with screw cock
K: Glass tube for bumping prevention

(ii) Procedure: Take 0.10 g of L-Carbocisteine in a vacuum distilling flask A, add 70 mL of water and 1 g of magnesium oxide, and connect the flask with a vacuum distilling apparatus. Place 5 mL of a solution of boric acid (1 in 200) as an absorbing liquid in receiver B, immerse the end of the branch of the vacuum distilling flask in the absorbing liquid, and distill in a water bath of 60°C, adjusting the degree of vacuum so that the distillatory rate is between 1 mL and 2 mL per minute, until 40 mL of distillate is obtained. During distillation, cool the bulb of receiver B with water. Lift the end of the branch from the absorbing liquid, rinse the end with a small amount of water, and add water to make exactly 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 2.0 mL of Standard Ammonium Solution in vacuum distilling flask A, and proceed in the same manner as the preparation of the test solution (not more than 0.02%).

(4) Heavy metals—Proceed with 1.0 g of L-Carbocisteine 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).

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

(6) Other amino acids—Dissolve 0.30 g of L-Carbocisteine in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 1 mL of this solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 10 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, in 15 mm length along the starting line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°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 spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying Not more than 0.30% (1 g, 105°C, 2 hours).

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

Assay Weigh accurately about 0.25 g of L-Carbocisteine, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS and 50 mL of acetic acid (100), and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 17.920 mg of $C_5H_9NO_4S$

Containers and storage Containers—Tight containers.