um nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(6) Related substances—Dissolve 0.050 g of Deferoxamine Mesilate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 50 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 of both solutions by the automatic integration method: the total area of all peaks other than the peak of deferoxamine from the sample solution is not larger than the peak area of deferoxamine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.32 g of diammonium hydrogenphosphate, 0.37 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.08 g of sodium 1-heptanesulfonate in 950 mL of water. Adjust this solution with phosphoric acid to a pH of 2.8, and to 800 mL of this solution add 100 mL of 2-propanol.

Flow rate: Adjust the flow rate so that the retention time of deferoxamine is about 15 minutes.

Selection of column: Dissolve 0.016 g of Deferoxamine Mesilate and 4 mg of methyl parahydroxybenzoate in 50 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 deferoxamine and methyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of deferoxamine from 20 μL of the standard solution is between 5 mm and 20 mm.

Time span of measurement: About twice as long as the retention time of deferoxamine after the solvent peak.

Water Not more than 2.0% (0.2 g, direct titration).

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

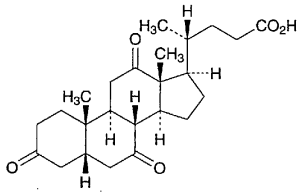
Assay Weigh accurately about 0.06 g of Deferoxamine Mesilate and Deferoxamine Mesilate Reference Standard (determine the water content before using in the same manner as Deferoxamine Mesilate), dissolve each in 20 mL of water, add 10 mL of 0.05 mol/L sulfuric acid TS, and add water to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of 0.05 mol/L sulfuric acid TS and exactly 0.2 mL of iron (III) chloride TS, then add water to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under the Ultraviolet-visible Spectrophotometry, using a solution prepared by adding 0.05 mol/L sulfuric acid TS to 0.2 mL of iron (III) chloride TS to make exactly 50 mL as the blank, and determine the absorbances, A_T and A_S, of each solution from the sample solution and the standard solution at 430 nm.

Amount (mg) of $C_{25}H_{48}N_6O_8.CH_4O_3S$
= amount (mg) of Deferoxamine Mesilate Reference Standard, calculated on the anhydrous basis
$\times \frac{A_T}{A_S}$

Containers and storage Containers—Tight containers.

Dehydrocholic Acid

デヒドロコール酸



$C_{24}H_{34}O_5$: 402.52
3,7,12-Trioxo-5β-cholan-24-oic acid [*81-23-2*]

Dehydrocholic Acid, when dried, contains not less than 98.5% of $C_{24}H_{34}O_5$.

Description Dehydrocholic Acid occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification **(1)** Dissolve 5 mg of Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and a blue-green fluorescence.

(2) To 0.02 g of Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation $[\alpha]_D^{20}$: +29 - +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point 233 - 242°C

Purity **(1)** Odor—To 2.0 g of Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—To 0.10 g of Dehydrocholic Acid, previously powdered in a mortar, add 30 mL of ethanol (95), and dissolve by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride—To 2.0 g of Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare

the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals—Proceed with 1.0 g of Dehydrocholic 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) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes. Cool, filter, and wash with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition Not more than 0.20% (1 g).

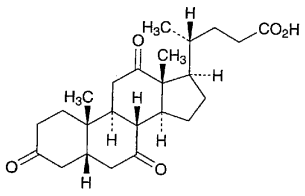
Assay Weigh accurately about 0.5 g of Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Well-closed containers.

Purified Dehydrocholic Acid

精製デヒドロコール酸



$C_{24}H_{34}O_5$: 402.52
3,7,12-Trioxo-5β-cholan-24-oic acid [*81-23-2*]

Purified Dehydrocholic Acid, when dried, contains not less than 99.0% of $C_{24}H_{34}O_5$.

Description Purified Dehydrocholic Acid occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Purified Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and blue-green fluorescence.

(2) To 0.02 g of Purified Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation $[\alpha]_D^{20}$: +29 - +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point 237 - 242°C

Purity (1) Odor—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—Dissolve 0.10 g of Purified Dehydrocholic Acid, previously powdered in a mortar, in 30 mL of ethanol (95) by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals—Proceed with 1.0 g of Purified Dehydrocholic 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) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes, cool, filter, and wash the filter with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition Not more than 0.20% (1 g).

Assay Weigh accurately about 0.5 g of Purified Dehydrocholic Acid , previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, then titrate with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Well-closed containers.