

(4) Related substances—Conduct this procedure without exposure to daylight, using a light-resistant vessel. Dissolve 0.10 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20  $\mu$ L 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 these solutions by the automatic integration method: the total area of peaks other than the peak of menatetrenone from the sample solution is not larger than the peak area of menatetrenone from the standard solution.

**Operating conditions—**

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

Time span of measurement: About 6 times as long as the retention time of menatetrenone after solvent peak.

**System suitability—**

Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20  $\mu$ L of this solution is equivalent to 7 to 13% of that of menatetrenone obtained from 20  $\mu$ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of menatetrenone is not more than 1.0%.

**Water** Not more than 0.5% (0.5 g, volumetric titration, direct titration).

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

**Assay** Conduct this procedure without exposure to daylight, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone Reference Standard (separately, determine the water in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution. Perform the test with 20  $\mu$ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios,  $Q_T$  and  $Q_S$ , of the peak area of menatetrenone to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of } C_{31}H_{40}O_2 \\ &= \text{amount (mg) of Menatetrenone Reference} \\ & \quad \text{Standard, calculated on the dehydrated basis} \\ & \quad \times \frac{Q_T}{Q_S} \end{aligned}$$

**Internal standard solution—**A solution of phytonadione in 2-propanol (1 in 20,000).

**Operating conditions—**

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

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

Mobile phase: Methanol

Flow rate: Adjust the flow rate so that the retention time of menatetrenone is about 7 minutes.

**System suitability—**

System performance: When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

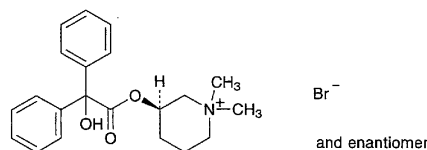
System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

## Mepenzolate Bromide

臭化メペンゾラート



$C_{21}H_{26}BrNO_3$ : 420.34

(*RS*)-3-(Hydroxydiphenylacetoxy)-1,1-dimethylpiperidinium bromide [76-90-4]

Mepenzolate Bromide, when dried, contains not less than 98.5% of mepenzolate bromide ( $C_{21}H_{26}BrNO_3$ ).

**Description** Mepenzolate Bromide is white to pale yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

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

Melting point: about 230°C (with decomposition).

**Identification (1)** To 0.03 g of Mepenzolate Bromide add 10 drops of sulfuric acid: a red color develops.

(2) Dissolve 0.01 g of Mepenzolate Bromide in 20 mL of water and 5 mL of dilute hydrochloric acid, and to 5 mL of this solution add 1 mL of Dragendorff's TS: an orange precipitate is produced.

(3) Determine the absorption spectrum of a solution of Mepenzolate Bromide in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of ab-

sorption at the same wavelengths.

(4) Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of water and 3 mL of nitric acid by heating. This solution responds to the Qualitative Tests for Bromide.

**Purity (1) Heavy Metals**—Proceed with 1.0 g of Mepenzolate Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not less than 20 ppm).

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

(3) **Related substances**—Dissolve 0.40 g of Mepenzolate Bromide in exactly measured 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 (1). Separately, dissolve 0.040 g of benzophenone in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10  $\mu$ L each of the sample solution, the standard solution (1) and the standard solution (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol, water and acetic acid (100) (3:3:2:1) to a distance of about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

**Loss on drying** Not more than 0.5% (1 g, 105°C, 4 hours).

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

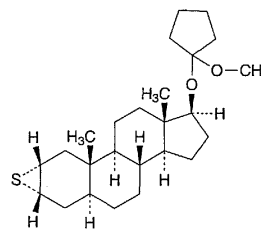
**Assay** Weigh accurately about 0.35 g of Mepenzolate Bromide, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 42.03 \text{ mg of } C_{21}H_{26}BrNO_3 \end{aligned}$$

**Containers and storage** Containers—Tight containers.

## Mepitiostane

メピチオスタン



$C_{25}H_{40}O_2S$ : 404.65

2 $\alpha$ ,3 $\alpha$ -Epithio-17 $\beta$ -(1-methoxycyclopentyl)-5 $\alpha$ -androstane [21362-69-6]

Mepitiostane contains not less than 96.0% and not more than 102.0% of  $C_{25}H_{40}O_2S$ , calculated on the anhydrous basis.

**Description** Mepitiostane occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

**Identification (1)** Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

(2) Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt between 144°C and 149°C.

(3) Determine the infrared absorption spectrum of Mepitiostane 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**  $[\alpha]_D^{20}$ : +20 – +23° (0.1 g, chloroform, 10 mL, 100 mm).

**Purity (1) Clarity and color of solution**—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

(2) **Heavy metals**—Proceed with 1.0 g of Mepitiostane 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 steroids**—Dissolve 0.020 g of Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution.