

Test solution. To 5 g of the freshly crushed drug, add 20 ml of *methanol R*. Stir for 15 min and filter.

Reference solution. Dissolve 5 mg of *chrysanthemin R* in 10 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, butanol R* (16:19:65 V/V/V).

Application: 10 µl, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: examine in daylight.

Results: see below the sequence of the zones present in the chromatograms obtained with the reference solution and the test solution.

Top of the plate	
Chrysanthemin: a violet-red zone	A violet-red zone
	A principal violet-red zone
	A compact set of other principal zones:
	– a violet-red zone
	– several violet-blue zones
Reference solution	Test solution

TESTS

Total ash (2.4.16): maximum 0.6 per cent.

Loss on drying (2.2.32): 80.0 per cent to 90.0 per cent, determined on 5.000 g of the freshly crushed drug by drying in an oven at 105 °C.

ASSAY

Crush 50 g extemporaneously. To about 5.00 g of the crushed, accurately weighed drug, add 95 ml of *methanol R*. Stir mechanically for 30 min. Filter into a 100.0 ml volumetric flask. Rinse the filter and dilute to 100.0 ml with *methanol R*. Prepare a 50-fold dilution of this solution in a 0.1 per cent V/V solution of *hydrochloric acid R* in *methanol R*.

Measure the absorbance (2.2.25) of the solution at 528 nm, using a 0.1 per cent V/V solution of *hydrochloric acid R* in *methanol R* as the compensation liquid.

Calculate the percentage content of anthocyanins, expressed as cyanidin-3-glucoside chloride, from the expression:

$$\frac{A \times 5000}{718 \times m}$$

718 = specific absorbance of cyanidin-3-glucoside chloride at 528 nm,

A = absorbance at 528 nm,

m = mass of the substance to be examined in grams.

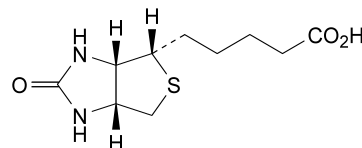
STORAGE

When frozen, store at or below – 18 °C.

01/2008:1073
corrected 6.0

BIOTIN

Biotinum



C₁₀H₁₆N₂O₃S
[58-85-5]

M_r 244.3

DEFINITION

Biotin contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder or colourless crystals, very slightly soluble in water and in alcohol, practically insoluble in acetone. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A.

Second identification: B, C.

- Examine by infrared absorption spectrophotometry (2.2.24), comparing with the spectrum obtained with *biotin CRS*.
- Examine the chromatograms obtained in the test for related substances (see Tests). The principal spot in the chromatogram obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).
- Dissolve about 10 mg in 20 ml of *water R* with heating. Allow to cool. Add 0.1 ml of *bromine water R*. The bromine water is decolourised.

TESTS

Solution S. Dissolve 0.250 g in a 4 g/l solution of *sodium hydroxide R* and dilute to 25.0 ml with the same alkaline solution.

Appearance of solution. Solution S is clear (2.2.1) and colourless (2.2.2, *Method II*).

Specific optical rotation (2.2.7). The specific optical rotation is + 89 to + 93, determined on solution S and calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (2.2.27), using as the coating substance a suitable silica gel (5 µm). *Prepare the solutions immediately before use and keep protected from bright light.*

Test solution (a). Dissolve 50 mg of the substance to be examined in *glacial acetic acid R* and dilute to 10 ml with the same solvent.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *glacial acetic acid R*.

Reference solution (a). Dissolve 5 mg of *biotin CRS* in *glacial acetic acid R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dilute 1 ml of test solution (b) to 20 ml with *glacial acetic acid R*.

Reference solution (c). Dilute 1 ml of test solution (b) to 40 ml with *glacial acetic acid R*.

Apply to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 5 volumes of *methanol R*, 25 volumes of *glacial acetic acid R* and 75 volumes of *toluene R*. Dry the plate in a current of warm air. Allow to cool and spray with *4-dimethylaminocinnamaldehyde solution R*. Examine immediately in daylight. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (c) (0.25 per cent).

Heavy metals (2.4.8). 1.0 g complies with limit test C for heavy metals (10 ppm). Prepare the standard using 10 ml of *lead standard solution (1 ppm Pb) R*.

Loss on drying (2.2.32). Not more than 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (2.4.14). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

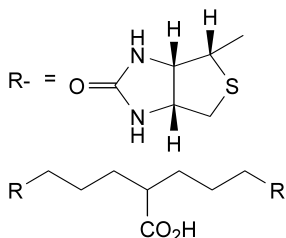
Suspend 0.200 g in 5 ml of *dimethylformamide R*. Heat until the substance has dissolved completely. Add 50 ml of *ethanol R* and titrate with 0.1 M *tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.2.20).

1 ml of 0.1 M *tetrabutylammonium hydroxide* is equivalent to 24.43 mg of C₁₀H₁₆N₂O₃S.

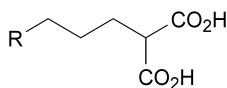
STORAGE

Store protected from light.

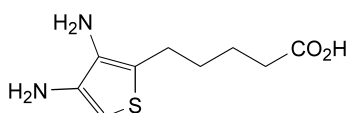
IMPURITIES



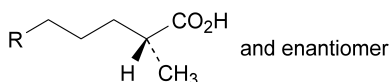
A. di[3-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]propyl]acetic acid,



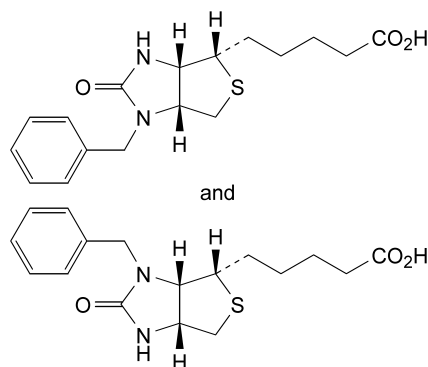
B. 4-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]butane-1,1-dicarboxylic acid,



C. 5-(3,4-diamino-2-thienyl)pentanoic acid,



D. 2-methyl-5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid,

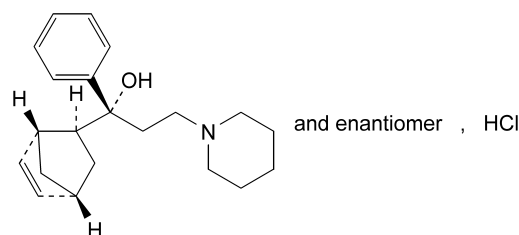


E. 5-[(3a*S*,4*S*,6a*R*)-3-benzyl-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid and 5-[(3a*S*,4*S*,6a*R*)-1-benzyl-2-oxohexahydrothieno[3,4-*d*]imidazol-4-yl]pentanoic acid.

01/2008:1074
corrected 6.0

BIPERIDEN HYDROCHLORIDE

Biperideni hydrochloridum



C₂₁H₃₀ClNO
[1235-82-1]

*M*_r 347.9

DEFINITION

(1*RS*)-1-[(1*RS*,2*SR*,4*RS*)-Bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water and in alcohol, very slightly soluble in methylene chloride.

mp: about 280 °C, with decomposition.

IDENTIFICATION

First identification: A, D.

Second identification: B, C, D.

A. Infrared absorption spectrophotometry (2.2.24).

Comparison: *biperiden hydrochloride CRS*.

B. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 25 mg of the substance to be examined in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (a). Dissolve 25 mg of *biperiden hydrochloride CRS* in *methanol R* and dilute to 5 ml with the same solvent.

Reference solution (b). Dissolve 5 mg of *biperiden impurity A CRS* in reference solution (a) and dilute to 2 ml with the same solution.

Plate: TLC silica gel *F*₂₅₄ plate *R*.