



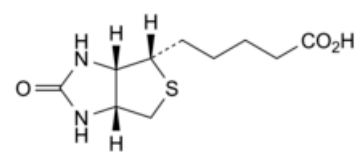
Edition: BP 2025 (Ph. Eur. 11.6 update)

Biotin



General Notices

(Ph. Eur. monograph 1073)



C₁₀H₁₆N₂O₃S 244.3 58-85-5

Action and use

Vitamin.

Ph Eur

DEFINITION

5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Very slightly soluble in water and in ethanol (96 per cent), practically insoluble in acetone. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

First identification: A.

Second identification: B.

A. Infrared absorption spectrophotometry ([2.2.24](#)).

Comparison [biotin CRS](#).

B. Thin-layer chromatography ([2.2.27](#)).

Prepare the solutions immediately before use and keep protected from bright light.

Test solution Dissolve 5 mg of the substance to be examined in [glacial acetic acid R](#) and dilute to 10 mL with the same solvent.

Reference solution Dissolve 5 mg of [biotin CRS](#) in [glacial acetic acid R](#) and dilute to 10 mL with the same solvent.

Plate [TLC silica gel plate R](#) (5 µm).

Mobile phase [methanol R](#), [glacial acetic acid R](#), [toluene R](#) (5:25:75 V/V/V).

Application 10 µL.

Development Over 3/4 of the plate.

Drying In a current of warm air.

Detection Allow to cool and spray with [4-dimethylaminocinnamaldehyde solution R](#); examine immediately in daylight.

Results The principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

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](#))

+ 89 to + 93 (dried substance), determined on solution S.

Related substances

Liquid chromatography ([2.2.29](#)). *Prepare the solutions immediately before use and keep protected from bright light.*

Solvent mixture [water R](#), [acetonitrile R](#) (50:50 V/V).

Test solution Dissolve 50 mg of the substance to be examined in the solvent mixture using sonication and dilute to 50.0 mL with the solvent mixture.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 10.0 mL with the solvent mixture.

Reference solution (b) Dissolve 5 mg of [biotin for system suitability CRS](#) (containing impurities A, C and E) in the solvent mixture and dilute to 5 mL with the solvent mixture.

Column:

— size: $l = 0.25$ m, $\varnothing = 4.6$ mm;

— stationary phase: [end-capped octadecylsilyl silica gel for chromatography R](#) (5 µm);

— temperature: 30 °C.

Mobile phase:

— mobile phase A: [methanesulfonic acid R](#), [acetonitrile R1](#), [water for chromatography R](#) (1:25:1000 V/V/V);

— mobile phase B: [methanesulfonic acid R](#), [water for chromatography R](#), [acetonitrile R1](#) (1:25:1000 V/V/V);

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 5	95	5
5 - 20	95 → 0	5 → 100
20 - 28	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 200 nm from 0 to 5 min and at 210 nm from 5 to 28 min.

Injection 10 µL.

Identification of impurities Use the chromatogram supplied with [biotin for system suitability CRS](#) and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, C and E.

Relative retention With reference to biotin (retention time = about 12 min): bromide = about 0.2; impurity C = about 0.25; impurity A = about 1.1; impurity E = about 1.3.

System suitability Reference solution (b):

— [resolution](#): minimum 1.5 between the peaks due to biotin and impurity A.

Calculation of percentage contents:

— *correction factor*: multiply the peak area of impurity E by 0.2;

— for each impurity, use the concentration of biotin in reference solution (a).

Limits:

— *impurities A, E*: for each impurity, maximum 0.5 per cent;

— *impurity C*: maximum 0.2 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 2.0 per cent;

— *reporting threshold*: 0.05 per cent, disregard the peak due to the bromide ion.

[Loss on drying \(2.2.32\)](#)

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

[Sulfated ash \(2.4.14\)](#)

Maximum 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.

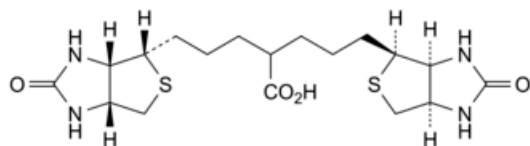
STORAGE

Protected from light.

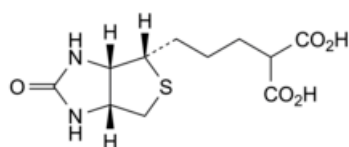
IMPURITIES

Specified impurities A, C, E.

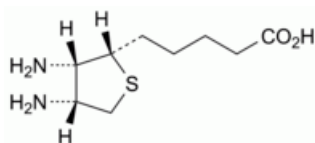
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph [Substances for pharmaceutical use \(2034\)](#). It is therefore not necessary to identify these impurities for demonstration of compliance. See also [5.10. Control of impurities in substances for pharmaceutical use](#)) B, D, F, G, H.



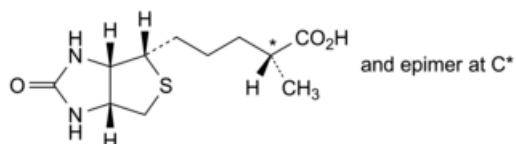
A. 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]-2-[[[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]propyl]pentanoic acid,



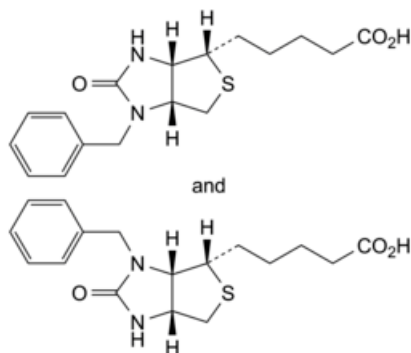
B. 4-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]butane-1,1-dicarboxylic acid,



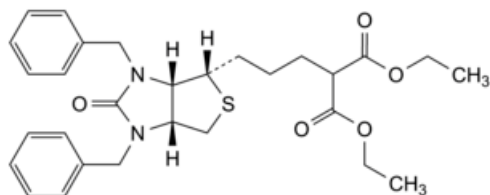
C. 5-[(2S,3S,4R)-3,4-diaminothioloan-2-yl]pentanoic acid,



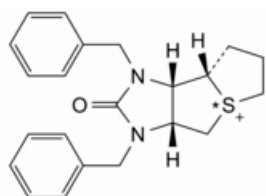
D. (2RS)-2-methyl-5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid,



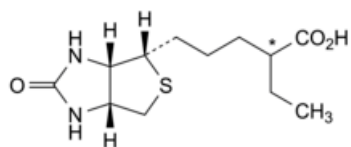
E. 5-[(3aS,4S,6aR)-1-benzyl-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid and 5-[(3aS,4S,6aR)-3-benzyl-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid,



F. diethyl 4-[(3aS,4S,6aR)-1,3-dibenzyl-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]butane-1,1-dicarboxylate,



G. (3aR,8aS,8bS)-1,3-dibenzyl-2-oxodecahydrothieno[1',2':1,2]thieno[3,4-*d*]imidazol-5-ium,



H. 2-ethyl-5-[(3aS,4S,6aR)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid.

Ph Eur