(6R,S)-5-Formyl-5,6,7,8-tetrahydropteroic acid

Product number 16.280 CAS number 4349-43-3

As starting material for the synthesis of (6R,S)-5-formyl-5,6,7,8-tetrahydropteroic acid we used (6R,S)-5-formyl-5,6,7,8-tetrahydrofolic acid, calcium salt. The side chain of (6R,S)-5-formyl-5,6,7,8-tetrahydrofolic acid is cleaved by bacteria ATCC 29861.
Since we had no bioreactor, we used a plastic basin with a diameter of about 40 cm. In this basin we gave 5 l of water and 400 ml of a culture medium. The culture medium had the following composition:
3.0 g KH_2PO_4
5.5 g K_2HPO_4
6.0 g $MgSO_4 . 7 H_2O$
400 mg $CaCl_2$
300 mg $FeSO_4 . 7 H_2O$
90 mg $MnSO_4 . H_2O$
90 mg $MoNa_2O_4 . H_2O$
30 ml of a fertilizer for hydroponics
4 l tap water

To the basin is added 60 g of (6R,S)-5-formyl-5,6,7,8-tetrahydrofolic acid, calcium salt and a small amount of the bacteria. In the following batches we added 5 ml of the reaction solution of the preceding batch.
The level of the liquid is marked with a felt-tip pen and the evaporated water is occasionally replaced with ion-free water. We kept the plastic basin in an utility shed, since the reaction solution stinks.
The temperature should be over 15°C.
The solution is stirred with a plastic rod and adjusted to pH 7.5 by adding 2 N NaOH.
The pseudomonas bacteria need oxygen, thus the liquid in the basin is stirred every third day and the pH is kept in the range between 7.3 and 8.0 by the addition of diluted phosphoric acid.
The reaction is finished when the pH does not raise anymore (after about 6 weeks).

The progress of the reaction can be observed by TLC.
A small sample must be filtered and diluted with water.
TLC foil: cellulose 400 UV254
The reference standards are dissolved in water by adding 1 drop of NH_3.
Developing solvent: 5% NH_4HCO_3
The spots can be visualized by using ultraviolet light.

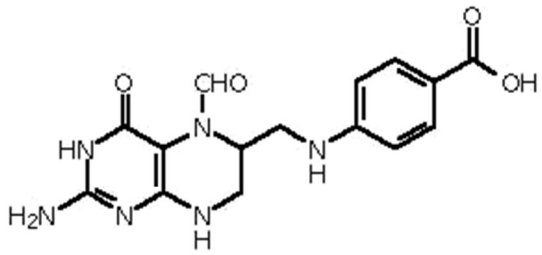
Diluted phosphoric acid is added until a pH of 6 is reached.
After 20 hours the crude (6R,S)-5-formyl-5,6,7,8-tetrahydropteroic acid is filtered through a 32 cm filtering funnel. *Please see the "General instructions for working with pteridines"*. The first 500 ml of the filtrate may be turbid and are filtered once more. The filtration proceeds very slowly. A tap is installed at the suction bottle, so that the filtration can run overnight. The filter cake is rinsed with 1 l of water. The filter cakes must well be sucked out and compacted with a glass stopper.

Recrystallisation
1/3* of the wet, crude (6R,S)-5-formyl-5,6,7,8-tetrahydropteroic acid is placed in a 10 l round bottom flask containing a magnetic stir bar and 4 l of water. 4 N NaOH is added until the (6R,S)-5-formyl-5,6,7,8-tetrahydropteroic acid is dissolved (pH about 11.0).
The mixture is filtered through a 32 cm filtering funnel. The filter may clog*, please see the *"General instructions for working with pteridines".*
The filter cake is rinsed with 300 ml of water. To the filtrate is added slowly with stirring through a dropping funnel diluted HCl until a pH of 4.5 is reached. After 20 hours the precipitated (6R,S)-5-formyl-5,6,7,8-tetrahydropteroic acid is filtered through a 32 cm filtering funnel. The filter cake is rinsed with 400 ml of water and then dried in a vacuum desiccator over NaOH.

Purity: 98.8% (HPLC)

Description: off white powder

Solubility: Soluble in water with the help of minimal ammonium hydroxide



(6R,S)-5-Formyl-5,6,7,8-tetrahydropteroic acid

$C_{15}H_{16}N_6O_4$
344.32
C 52.32% H 4.68% N 24.41% O 18.59%

Product no. 16.280

HPLC conditions:

Sample: 1 mg/ml 0.01 N NaOH
Column: Spherisorb S5 ODS1, 150 mm x 4.6 mm
Eluant: 10 mM Na_2HPO_4 pH 7.0 + 10% MeOH
Flow: 1 ml/min
Detection: 254 nm