General instructions for working with pteridines

Number 09.010

Pteridines are derivatives of the parent heterocycle pteridine. These include biologically important molecules such as folic acid and biopterin.

Filtration
For small amounts we used Whatman® glass microfiber filtration funnels with Whatman® glass microfiber filters or filter papers.
When active carbon is filtered out from solutions containing pteridines the active carbon may pass through the filter. When from basic solutions the active carbon is filtered out, the filter cake has to be rinsed with a basic solution. If it is washed with pure water, the active carbon often passes through the filter.
Some pteridines precipitate as very fine precipitates and clog the filter. In order to improve the speed of the filtration we put under the filter paper a plastic fabric with a diameter smaller than that of the filter paper.
The filtration proceeds often very slowly. A tap is installed at the suction bottle, so that the filtration can run overnight.
Fine precipitates lead to thick filter cakes. Such filter cakes must well be sucked out and compacted with a glass stopper.
The washing liquid is always added in at least 3 portions.

Evacuation
Some pteridines are very sensitive to oxygen and to prevent oxidation, solutions of these compounds should be evacuated.
We used two- and three-stage diaphragm pumps and a chemistry-hybrid-pump (a combination of a two-stage rotary vane pump and a two-stage chemistry diaphragm pump). All pumps must be warm before use and after use they must be run with open gas ballast.

Precipitations
Sometimes pteridines precipitate very slowly, especially when there are a lot of impurities in the solution. The formation of a precipitate may take 2 or 3 days or even longer. Mixing the suspension slightly from time to time enhances the rate of precipitation.
The cleaner the dissolved compound is, the faster it precipitates.

Hydrogenations
For the hydrogenation of small amounts of pteridines, we used a simple equipment.
To a three-neck round bottom flask were attached two rotaflow stopcocks.
A vacuum hose must be inserted into the mouth of a metallic balloon and sealed with a cord. Then the balloon is evacuated in order to test whether the connection is tight. This is not easy to achieve. It may help to coat the vacuum tube with silicone before to insert it.
The vacuum tube of the balloon is imposed on one of the stopcocks.
A strong egg-shaped stir bar is added.

In order to remove all air from the three-necked round bottom flask, the equipment is twice evacuated and filled with nitrogen.

Reductions using sodium dithionite Na2S2O4
The reaction speed depends strongly on the pH.
Details can be found in: Bernhard Schircks, "Neue regiospezifische Synthese von L-Biopterin und von dessen Derivaten" (Dissertation-Zurich, 1978)
Page 48 and 75.

HPLC
Many pteridines are sensitive to oxygen. Solutions of these pteridines are often oxidized before they could be injected.
Addition of DTT may improve the result.
When peaks of impurities appear, you have to find out whether these peaks are peaks of oxidation products. The easiest way is to inject the same sample again after an hour. Peaks of oxidation or degradation products will increase.
We recommend dissolving poorly soluble substances in 0.01N NaOH. NH3 solutions can produce a weak signal.

TLC
TLC with silica gel on polyester sheets: If lipophilic compounds are separated, the spots can be visualized by hot steam.
Some water is placed in a beaker and heated on a heating plate, but the water should not boil.
The TLC sheet is placed on the beaker and when it becomes slightly wet the spots can be seen.

Column chromatography
The solutions that are applied to the column often contain impurities that clog the top layer of the column. This causes that the column cannot run correctly. The top layer of the column must then be scratched.