106. Pterin Chemistry

Part 89[1])

Natural Primapterin Belongs to the L-Series of 7-(Polyhydroxypropyl)pterins[2])

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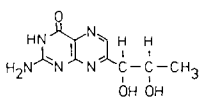
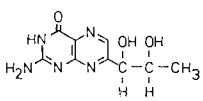
Natural primapterin (80 μg) was isolated from 200 ml of urine of a patient with mild hyperphenylalaninemia. The CD curve of the compound showed that it belongs to the L-series of 7-(polyhydroxypropyl)pterins.

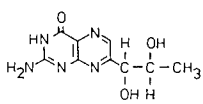
Primapterin has been isolated from the urine of a patient with mild hyperphenylalaninemia [3] and has been found in two other children with the same symptoms [4]. HPLC comparison with reference products showed that primapterin coeluated with 7-(L-*erythro*-1′,2′-dihydroxypropyl)pterin (**1**) already synthesized by *Green* and *Rembold* [5]. This structure, without stereochemical identification, has been confirmed by GC-MS [6].



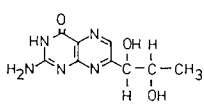
1 L-primapterin



2 D-primapterin



3 D-*threo*-primapterin



4 L-*threo*-primapterin

A 7-(1′,2′-dihydroxypropyl)pterin structure may occur in four different diastereoisomeric forms, *i.e.* **1**–**4**, the presence in the urine of two of which, namely **3** and **4**, were excluded by HPLC. But neither HPLC nor GC-MS can distinguish if the *erythro*-configuration of primapterin belongs to the D-*erythro* (**2**) or the L-*erythro* (**1**) series. To answer this question, we isolated *ca.* 80 μg of natural primapterin, and almost the same quantity of biopterin for comparison, from 200 ml of urine of a patient. CD curves of these compounds compared with those of synthetic substances showed that both belong to the L-series.

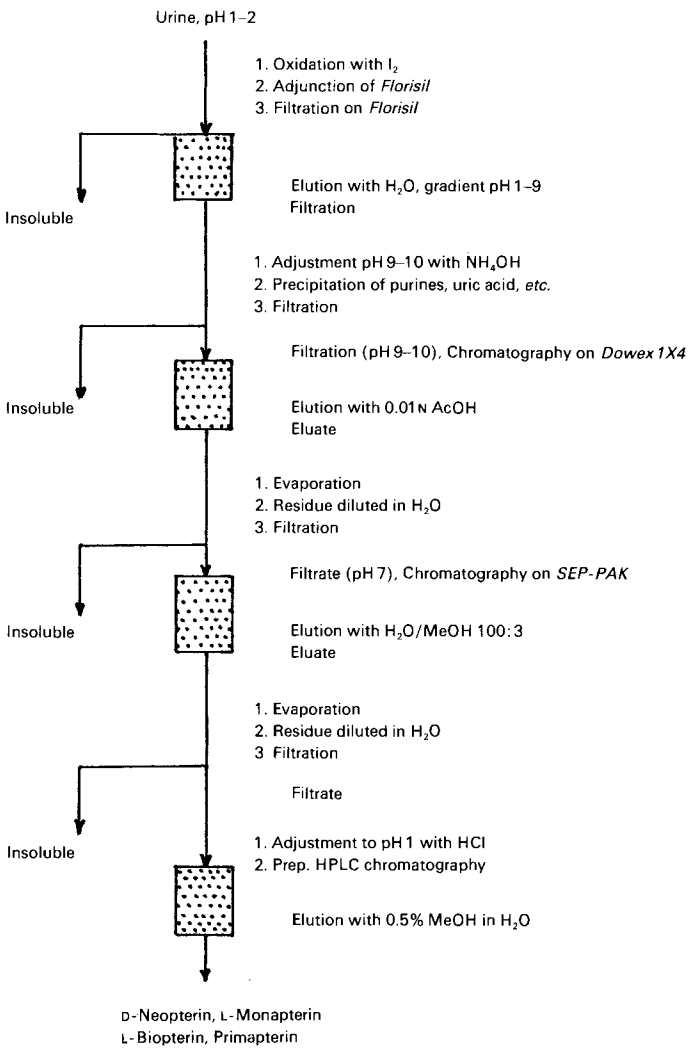
Experimental Part

1. *General.* Purification of the pterins from urine (*Scheme*) according to the outlines given in [3]; *Florisil* and *Dowex 1 × 4, Fluka AG* (CH–9470 Buchs); *SEP-PAK* C_{18} cartridges; anal. HPLC according to [7]; prep. HPLC according to *Matasovic* [8]; TLC: silica gel, *Merck 60* F_{254}; eluant: i-PrOH/3% boric acid soln. 4:1. CD: *Jasco-J-500A* instrument [9].

[1]) Presented at the 4th International Conference of Pteridines and Related Biogenic Amines, St. Moritz, March 3–8, 1990. Part 88: [1].

[2]) We use in this publication the designation rules for pterin derivatives proposed in [2].

Scheme



2. *Purification of Pterins.* In a beaker with magnetic stirrer, the pH of a patient's urine was adjusted to 2–3 with 1N HCl and oxidized with a 7% I_2 soln. in MeOH, avoiding an excess of I_2 (KI/starch-paper). Then *Florisil* was slowly added by stirring (r.t., 1 h) until the urine soln. was nearly colorless and showed a strong blue fluorescence. It was then divided into two portions of 100 ml, and each portion was applied to a *Florisil* column (13 × 30 mm) and washed with 0.1N HCl and H_2O. The pterins were eluated with the following soln.: 0.05N HCl, H_2O, 0.01N NH_4OH (pterin test with TLC). The slightly yellow soln. was brought to pH 9–10 with 2N NH_4OH. Purines and uric acid precipitated and were filtered off. The basic filtrate was applied to a *Dowex 1 × 4* column (26 × 40 mm) and washed with H_2O to neutrality. The filtrate must be free of pterins (TLC). Elution occurred with 0.01N AcOH. During this operation, pterins were separated from most salts, purines, uric acid, and other components of the urine. HPLC showed in the AcOH eluate D-neopterin, primapterin, and L-biopterin in almost equal concentration, a small quantity of L-monapterin, and traces of pterin. The compounds were not pure enough for prep. separation. Therefore, the soln. was evaporated once more. Then 4–5 ml H_2O were added to the residue and the insoluble parts

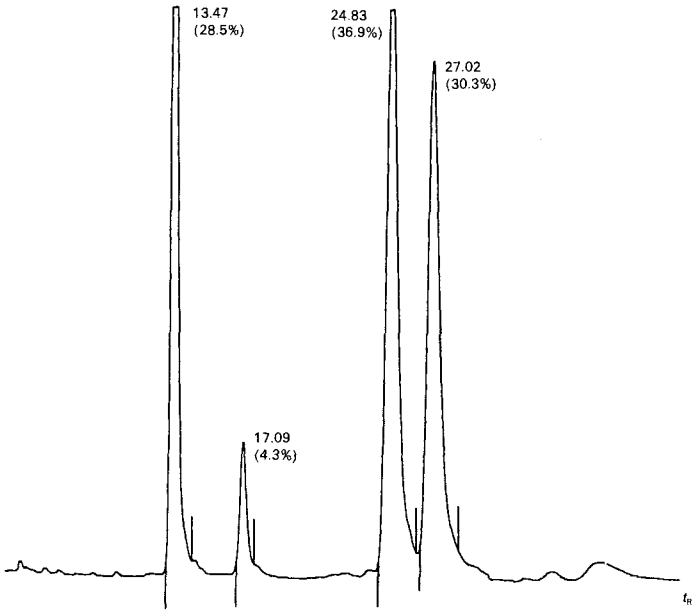


Fig. 1. *Anal. HPLC of the pterin solution ready for prep. HPLC.* 0.5% MeOH/H_2O.

filtered off. Fractions of 1 ml of the filtrate were purified by chromatography on *SEP-PAK* cartridges which were washed with H_2O/MeCN 4:1 (25 ml). Elution was carried out with 25 ml of H_2O/MeOH 100:3, and then 5 ml of H_2O/MeOH/i-PrOH 90:5:5. The collected eluates were evaporated, diluted in 7–8 ml H_2O, centrifuged for final elimination of the insoluble parts, adjusted to pH 1–2 with HCl and analyzed for pterins by prep. HPLC (*Fig. 1*): D-neopterin (28.5%), L-monapterin (4.3%), L-biopterin (36.9%), and primapterin (30.3%).

3. *Separation of the Single Pterins and Configuration of Primapterin.* The separation of the pterins was performed with fractions of 0.5 ml of liquid, applied to a prep. HPLC column *N°SP ODS 05 83 11 074*

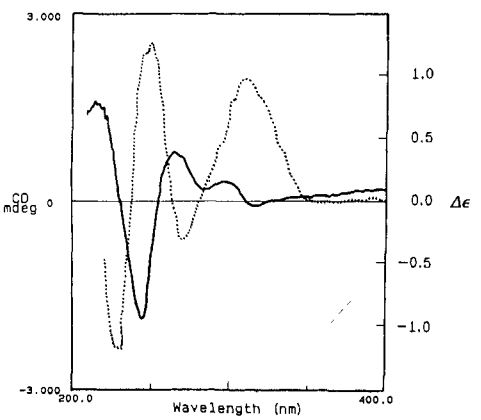


Fig. 2. *CD spectrum of* L-*biopterin isolated from urine* (–; *ca.* 80 μg in 4.5 ml of 0.1N HCl) *and mirror image of CD spectrum of synthetic* L-*biopterin* (···)

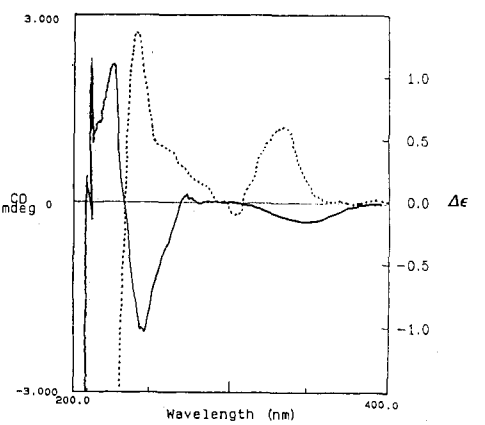


Fig. 3. *CD spectrum of primapterin isolated from urine* (–; *ca.* 80 μg in 4.5 ml of 0.1N HCl) *and mirror image of CD spectrum of synthetic* L-*primapterin* (···)

(20.5 × 250 mm; *Bischoff Analysentechnik*, CH–7250 Leonberg), with adsorbent *Spherisorb ODS*, 5 μ; eluant 0.5% MeOH, isocratic, flow 9 ml/min. After separation, the eluates of L-biopterin and primapterin were reduced i.v. to 4.5 ml, adjusted to pH 1, and the CD curves were measured and compared with the mirror image of the known curves of synthetic L-biopterin and L-primapterin [9]. The comparison showed distinctly that both isolated pterins belong to the L-series (*Figs. 2* and *3*).

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