96. Pterin Chemistry

Part 92[1])

Loading Experiments with 6α,β-Tetrahydro-L-[3′-$^2H_1$]biopterin

by **Carmen Adler, Hans-Christoph Curtius, Erich Wetzel,** and **Max Viscontini***

Division of Clinical Chemistry, Department of Pediatrics, University of Zurich, Steinwiesstr. 75, CH–8032 Zurich

and **Tullio A. Giudici**

Division of Genetics, Childrens Hospital, Los Angeles, USA

and **Milan Blaskovics**

Department of Pediatrics, Kaiser Permanente, Fontana, USA

and **Marie O. Rolland** and **Pierre Guibaud**

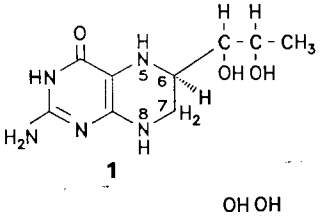
Centre d'Etude des Maladies Metaboliques, Hopital Debrousse, F–Lyon

(18.III.92)

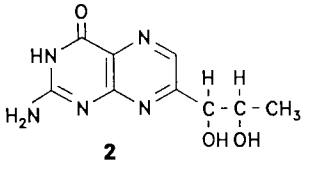
6α,β-Tetrahydro-L-[3′-$^2H_1$]biopterin ([3′-$^2H_1$]-**1**) was administered orally to two primapterinuric patients in order to investigate the biosynthetic pathway of 7-substituted pterins in humans. L-Primapterin (**2**) and L-biopterin were isolated from urine after loading and measured by GC/MS. L-Biopterin and L-primapterin were labelled with ^2H to an equal extent. From this result, one can conclude that L-primapterin is formed from tetrahydro-L-biopterin, very probably *via* an intramolecular rearrangement.

Introduction. – Since 7-substituted pterins have been detected in the urine of patients with a transient form of hyperphenylalaninemia [2] [3], *e.g.* 7-isomer of L-biopterin, referred to as L-primapterin (**2**) [4], and 7-isomer of D-neopterin, referred to as D- or L-anapterin (**4**) [5] (whose configuration has not been determined until now), the origin of these metabolites has not been known.

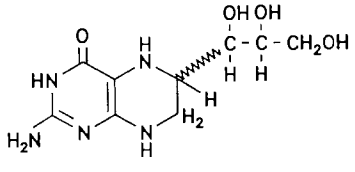
Three findings suggest that the 7-substituted pterins may be derived from their 6-substituted tetrahydro forms, *i.e.* 6β-tetrahydro-L-biopterin (**1**)[2]) and 6α,β-tetrahydro-



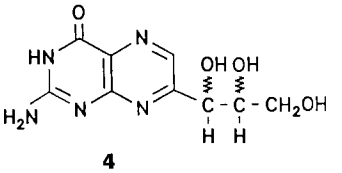
1



2



3



4

[1]) Presented at the '5th International Conference on Pteridines and Related Biogenic Amines and Folates', Cairns, November 16–22, 1991. Part 91: [1].

[2]) We use in this publication the designation rules for pterin derivatives proposed in [6].

D-neopterin (**3**). *1*) Oral loading of a primapterinuric patient with 6β-tetrahydro-L-biopterin (**1**) led to an increase of both L-biopterin and L-primapterin (**2**) in urine, while their ratio remained almost unchanged [7]. *2*) Determination of the absolute configuration by circular dichroism (CD) of natural primapterin showed that it belongs to the L-series, as does its 6-isomer biopterin [4]. *3*) *In vitro* incubation experiments of **1** or 6α,β-tetrahydro-D-neopterin (**3**) with pterin carbinolamine dehydratase-free rat or human phenylalanine hydroxylase resulted in the formation of L-primapterin, and D- or L-anapterin (**4**), respectively [8].

On the basis of these observations, we conclude that the formation of the 7-substituted pterins proceeds *via* their 6-isomers. To prove this hypothesis *in vivo*, we synthesized L-[3′-$^2H_1$]biopterin [9]. The reduction to its 6α,β-tetrahydro form was performed catalytically, and the mixture of 6α,β-tetrahydro-L-[3′-$^2H_1$]biopterin was loaded orally to two male primapterinuric patients (*T.S.*, 5 y and *P.P.*, 4 y) with a dose of 10 mg of [3′-$^2H_1$]-**1** per kg body weight. We purified and isolated L-biopterin and L-primapterin from *ca.* 200 ml urine before and after loading as described in the *Exper. Part.* The GC/MS analysis of these TMS derivatives were obtained to assess the extent of ^2H-incorporation after loading.

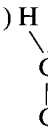
Results. – Mass-spectral fragmentation patterns of L-biopterin and L-primapterin isolated from urine of primapterinuric patients before loading were identical with each other and with MS data of synthetic compounds [5]. MS Data of L-biopterin and L-primapterin isolated after loading were also identical with those of synthesized, labelled L-biopterin [9]. The extent of deuteration of chemically synthesized, labelled L-biopterin was *ca.* 80%. The MS spectra of L-biopterin and L-primapterin, isolated after patient loading, displayed a 40% increase in signal intensity at m/z values, corresponding to ion structures expected to incorporate ^2H (*Figs. 1–4*).

MS Analysis of L-primapterin and L-biopterin was performed both before and after patient loading. Our definition of '% increase by deuteration' is the % difference in the m/z signals shifted by 1 Da before and after loading.

Three signals are of special importance for comparison:

1) $M^{+\cdot}$ before loading m/z 525, after loading m/z 525 and 526 (increase of intensity by deuteration: *ca.* 40%)

2) $[M - CH_3]^{+}$ before loading m/z 510, after loading m/z 510 and 511 (increase of intensity by deuteration: *ca.* 40%)

3)  before loading m/z 117

 after loading m/z 117 and 118 (increase of intensity by deuteration: *ca.* 40%)

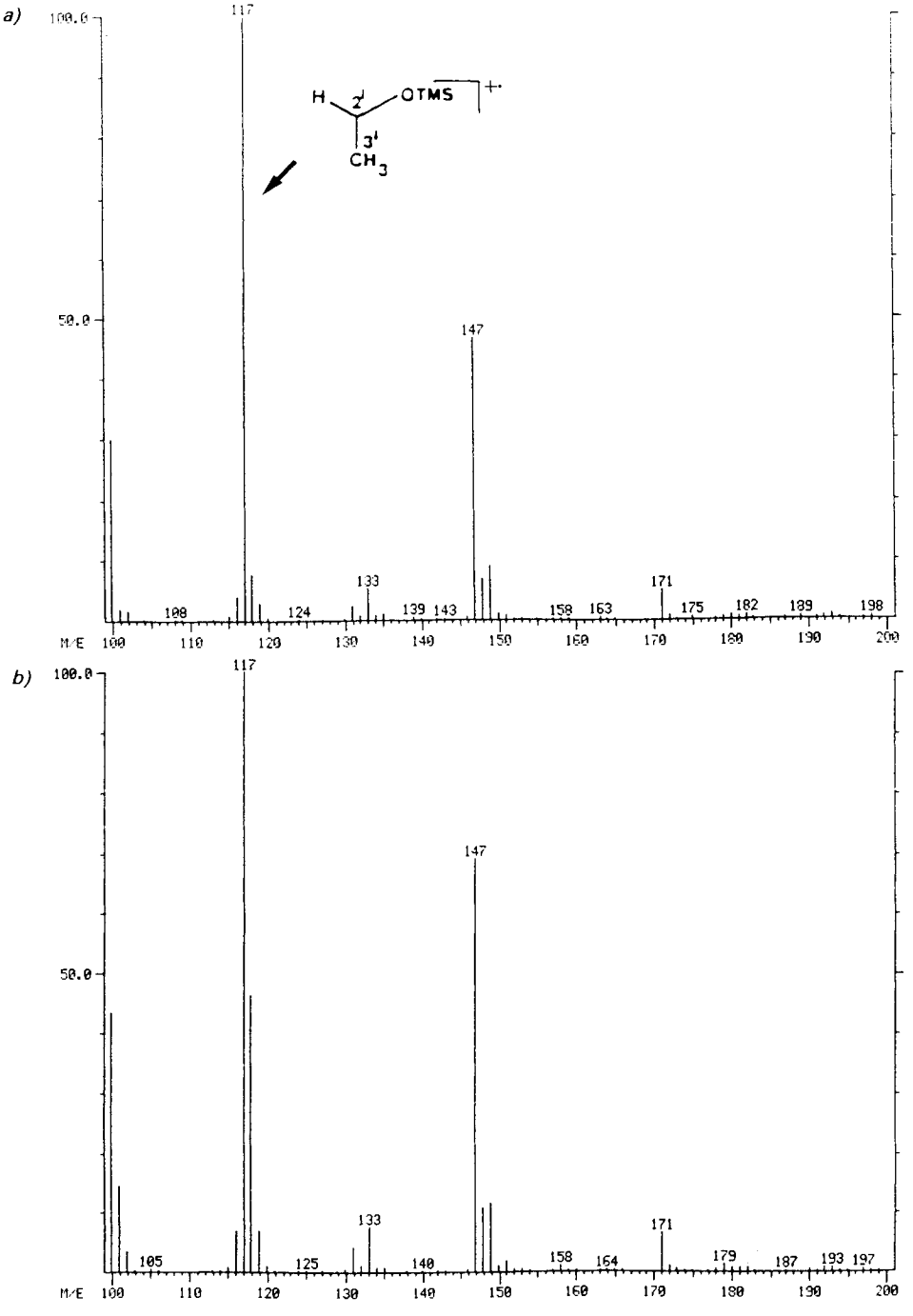


Fig. 1. *Low-molecular fragments of* L-*biopterin. a*) Reference compound; *b*) isolated from urine after loading with [3′-$^2H_1$]-**1**.

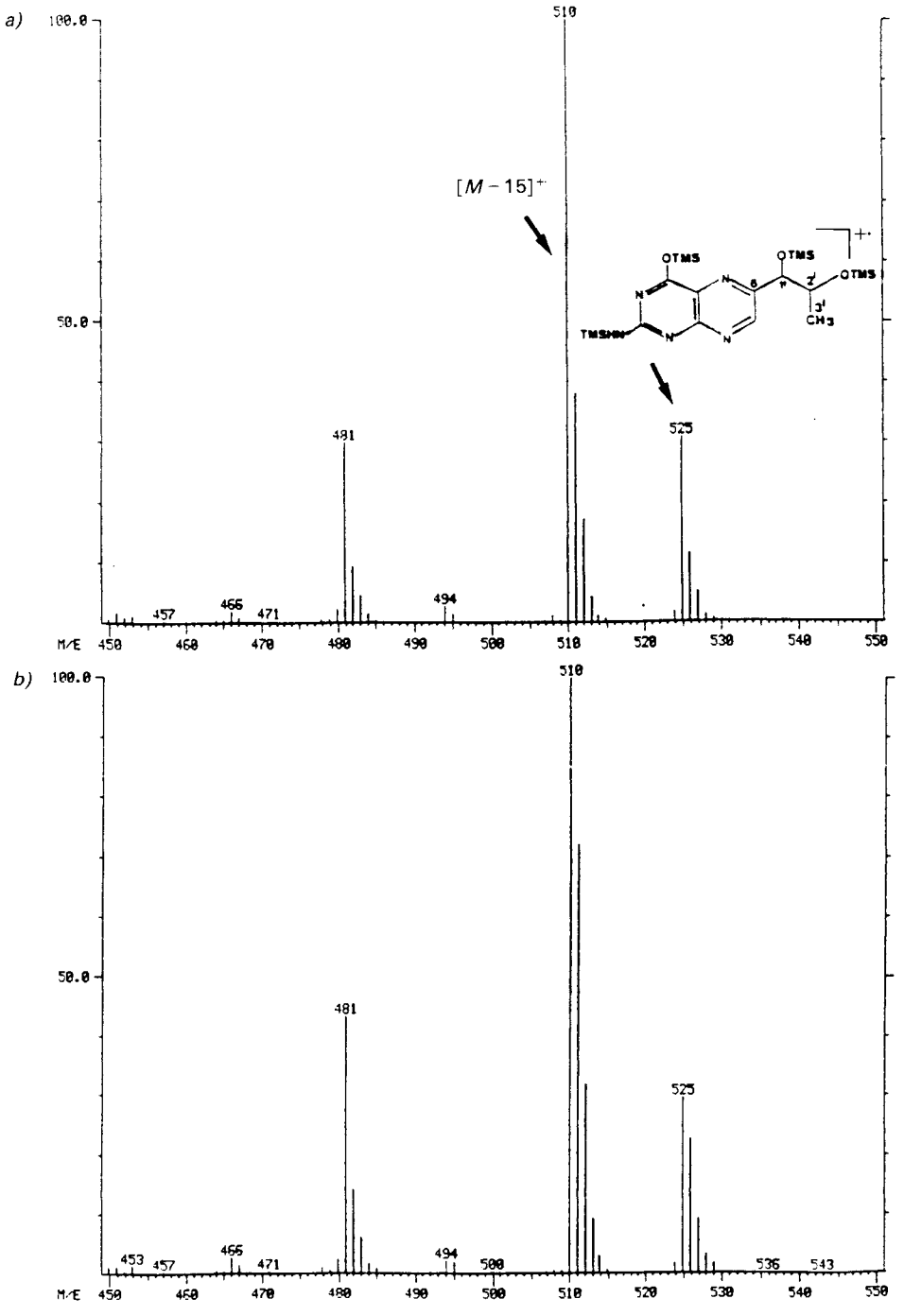


Fig. 2. *High-molecular fragments of* L-*biopterin. a*) Reference compound; *b*) isolated from urine after loading with [3′-$^2H_1$]-**1**.

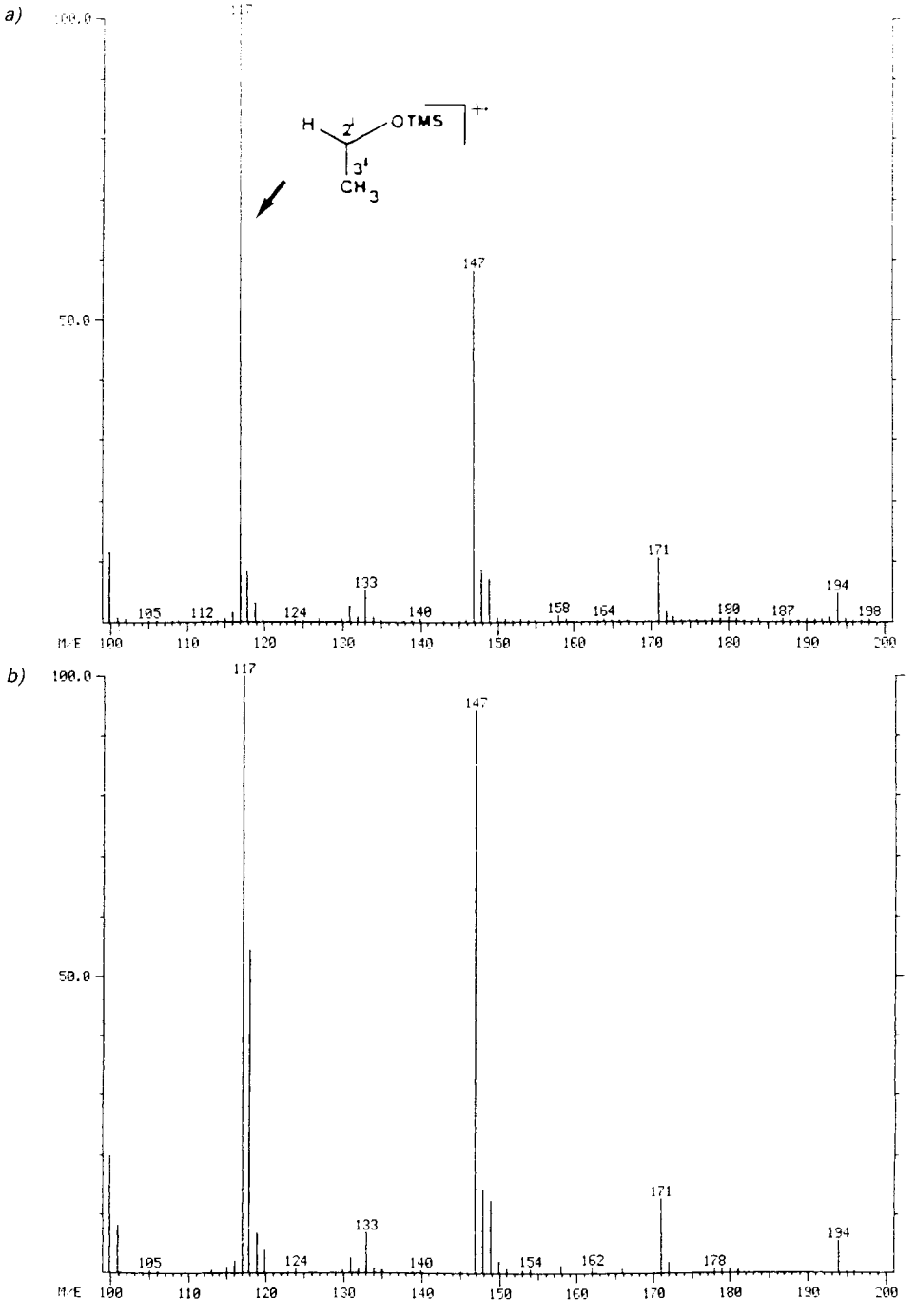


Fig.3. *Low-molecular fragments of* L-*primapterin. a*) Reference compound; *b*) isolated from urine after loading with [3′-$^2H_1$]-**1**.

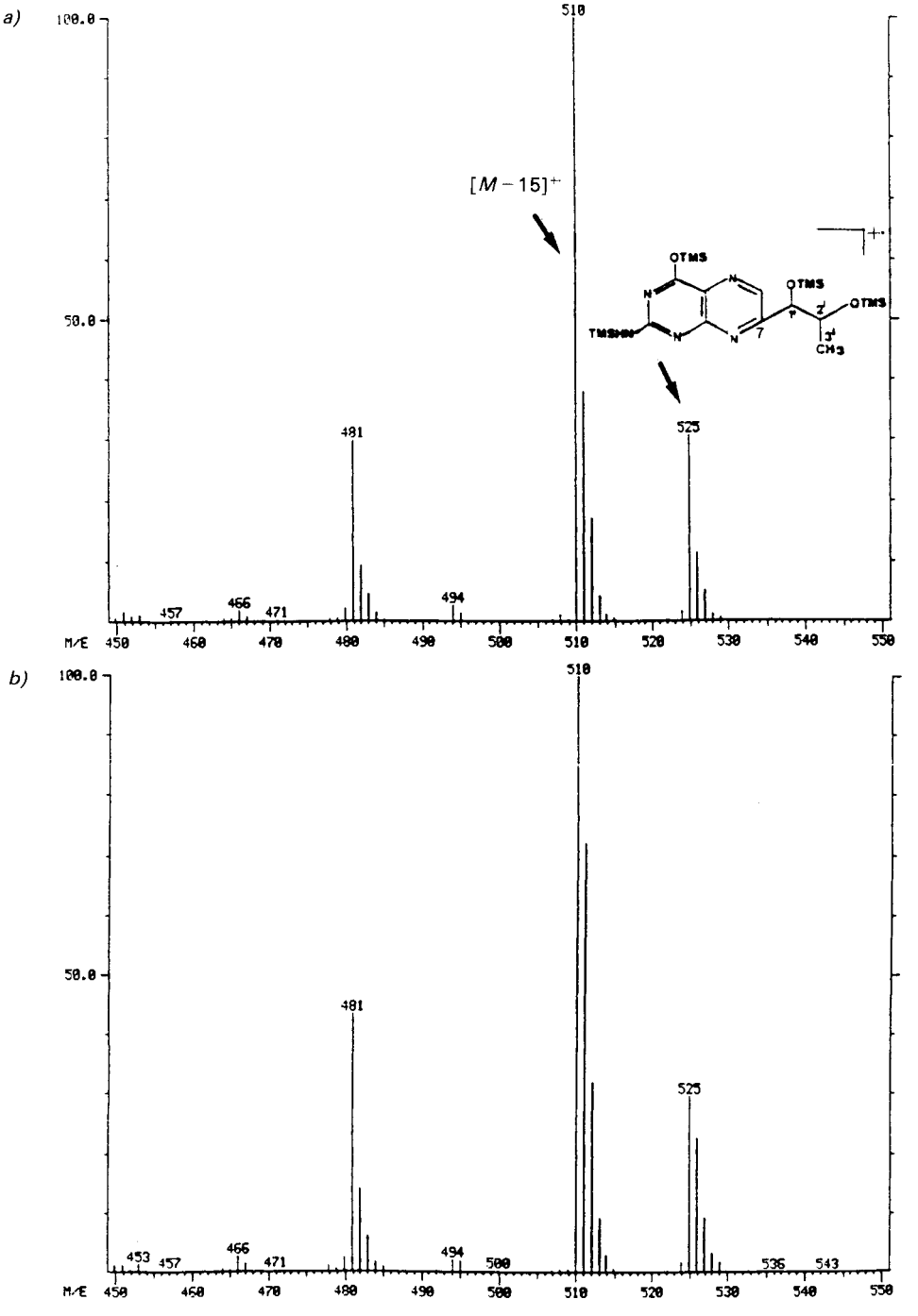


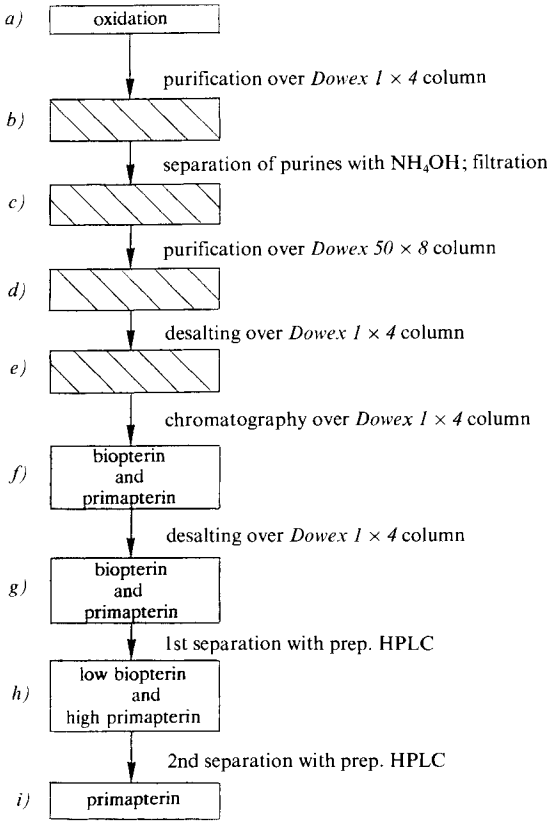
Fig. 4. *High-molecular fragments of* L-*primapterin. a*) Reference compound; *b*) isolated form urine after loading with [3′-$^2H_1$]-**1**.

All other fragment ions of L-biopterin and L-primapterin obtained before and after loading without ^2H remained unchanged in relative intensity. The fact that both isolated L-biopterin and L-primapterin show the same extent of deuteration indicates that L-primapterin may arise from an intramolecular rearrangement of tetrahydro-L-biopterin.

Experimental Part

1. *General. Dowex 1 × 4* (washed with 0.1N NaOH and H_2O before use) and *Dowex 50 × 8* (washed with 0.1N HCl and H_2O before use) from *Fluka AG* (CH-9470 Buchs). Anal. HPLC according to [10]; prep. HPLC analogous to anal. HPLC. MS: *VG-16F* single-focusing magnetic-field instrument; volatile derivatives of pterins obtained with bis(trimethylsilyl)trifluoroacetamide [11].

Scheme



2. *Purification and Separation of Pterins* (*Scheme*). *a*) The pH of 200 ml of urine was adjusted to 2–3 with 1N HCl and oxidized at 0–5° with a 7% I_2 soln. in MeOH, avoiding an excess of I_2 (KI/starch paper).

b) The acidic soln. was filtered through a filter paper and applied to a *Dowex 1 × 4* column (45 × 45 mm). Brown substances remained adsorbed on top of the column. Pterins and salts passed through, all the fractions with blue fluorescence were recovered (pterin test with anal. HPLC).

c) The slightly yellow soln. was brought to pH 9–10 with 2N NH_4OH. Purines and uric acid were precipitated and filtered off.

d) The basic filtrate was applied to a *Dowex 50 × 8* column (30 × 100 mm). All fluorescent fractions containing pterins passed through with salts and were collected.

e) For desalting, the fluorescent filtrate was made alkaline with 2N NH_4OH (200 ml of filtrate and 10 ml of 32% aq. NH_3) and applied to a *Dowex 1 × 4* column (45 × 100 mm). Pterins remained adsorbed at the top of the column and salts passed through. The column was washed with H_2O, and the pterins were eluted with 0.1N HCOOH.

f) The eluate was made alkaline with NH_4OH and applied to a *Dowex 1 × 4* column (20 × 200 mm). After adsorption of pterins and washing with little H_2O, pterins were fractionated by eluting with 0.1N $NH_4^+COO^-$ buffer, pH 7.8. The solns. were analyzed with HPLC. Fractions containing L-biopterin, L-primapterin, and small amounts of D-neopterin and pterin were collected. As salts are not adsorbed on the column, they are also present in the collected pterin fractions.

g) For desalting, a *Dowex 1 × 4* column (30 × 75 mm) was used. The alkaline pterin soln. (pH 9–10 with NH_4OH) was applied to the column. Salts passed through, and pterins remained adsorbed at the top of the column. The column was washed with H_2O, and pterins were aluted with 0.05N HCOOH. The blue fluorescent soln. was evaporated to 10 ml. HPLC showed that the formyl eluate contained low levels of D-neopterin and pterin, and high levels of L-biopterin and L-primapterin.

h) The separation of the pterins was performed with 0.1-ml fractions of liquid, applied to prep. HPLC column *N°SP ODS 05831 1074* (20.5 × 200 m; *Bischoff Analysentechnik*, CH–7250 Leonberg), with adsorbent ODS, 5 μ; eluent H_2O/MeOH 99:1, isocratic, flow 7.5–9.0 ml/min.

i) L-Primapterin fractions were concentrated to a volume of 5 ml. Because some L-biopterin was also present, a second prep. HPLC became necessary. After separation, the eluates of L-biopterin and L-primapterin were evaporated to dryness, and derivatized to their TMS ethers for GC/MS. Using the above method 300 μg of L-biopterin and 50 μg of L-primapterin were isolated from 200 ml of urine almost free form other substances.

This work was supported by the *Swiss National Science Foundation* (project No. 3.159–0.88 and 31-28797.90).

REFERENCES

[1] B. Fischer, J. Strähle, M. Viscontini, *Pteridines* **1992,** in press.

[2] J.-L. Dhondt, P. Guibaud, M.O. Rolland, C. Dorche, S. Andre, G. Forzy, J.M. Hayte, *Eur. J. Pediatr.* **1988,** *147,* 153.

[3] M. Blaskovics, T.A. Guidici, *New Engl. J. Med.* **1988,** *319,* 1611.

[4] M. Viscontini, *Helv. Chim. Acta* **1990,** *73,* 1064.

[5] H.-Ch. Curtius, T. Kuster, A. Matasovic, N. Blau, J.-L. Dhondt, *Biochem. Biophys. Res. Commun.* **1988,** *153,* 715.

[6] M. Viscontini, in 'Pteridines and Biogenic Amines in Neuropsychiatry, Pediatrics, and Immunology', Eds. R.A. Levine, S. Milstien, D.M. Kuhn, and H.-Ch. Curtius, Lakeshore Publishing Company, Grosse Pointe, Michigan, 1989, pp. 33–50.

[7] H.-Ch. Curtius, A. Matasovic, G. Schoedon, T. Kuster, P. Guibaud, T.A. Giudici, N. Blau, *J. Biol. Chem.* **1990,** *265,* 3923.

[8] H.-Ch. Curtius, C. Adler, I. Rebrin, C.W. Heizmann, S. Ghisla, *Biochem. Biophys. Res. Commun.* **1990,** *172,* 1060.

[9] C. Adler, H.-Ch. Curtius, S. Datta, M. Viscontini, *Helv. Chim. Acta* **1990,** *73,* 1058.

[10] A. Niederwieser, W. Staudenmann, E. Wetzel, in 'Biochemical and Clinical Aspects of Pteridines', Eds. H. Wachter, H.-Ch. Curtius, and W. Pfleiderer, Walter de Gruyter, Berlin–New York, 1982, Vol. 1, pp. 81–102.

[11] T. Lloyd, S. Markey, N. Weiner, *Anal. Biochem.* **1971,** *42,* 108; J.W. Serum, P. Haug, T. Urushibara, H.S. Forrest, *Z. Anal. Chem.* **1972,** *262,* 110; R. Weber, W. Frick, M. Viscontini, *Helv. Chim. Acta* **1974,** *57,* 1485; T. Kuster, A. Niederwieser, *J. Chromatogr.* **1983,** *278,* 245.