

Pteridines  
Vol. 3, pp. 25–28

### Short Communication

## 7-Substituted Pterins: Loading Experiments with 3'-Mono Deuterated Tetrahydro-L-Biopterin

C. Adler, H.-C. Curtius<sup>1)</sup>, E. Wetzel, T. A. Giudici\*, M. Blaskovics\*\* and M. Viscontini

Universitätskinderspital Zürich, Abteilung Klinische Chemie, Steinwiesenstr. 75, CH-8032 Zürich, Switzerland

\* Division of Genetics, Childrens Hospital, Los Angeles, U. S. A.

\*\* Department of Pediatrics, Kaiser Permanente, Fontana, U. S. A.

(Received March 1992)

### Introduction

Since 7-substituted pterins have been detected in the urine of patients with an atypical, transient form of hyperphenylalaninemia the origin of these metabolites has been unclear for a long time. The following pterins have been found so far: the 7-isomer of L-biopterin referred to as L-primapterin, the 7-isomer of D-neopterin referred to as D- or L-anapterin, and the 6-oxo-7-isomer of 7-oxo-L-biopterin referred to as 6-oxo-L- or D-primapterin (1).

Two findings make it plausible that these compounds derive from their 6-analogues: on the one hand, orally loading of a primapterinuric patient with L-tetrahydrobiopterin (L-BH<sub>4</sub>) led to an increase of both L-biopterin and L-primapterin (2). And, *in vitro* incubation of L-tetrahydrobiopterin with pterin-4a-carbinolamine dehydratase-free phenylalanine hydroxylase results in the formation of L-primapterin (3, 4). Analogue *in vitro* incubation experiments with D-tetrahydroneopterin also led to the formation of the 7-substituted isomer.

For *in vivo* investigations of L-primapterin biosynthesis we loaded a primapterinuric patient with side-chain mono-deuterium labelled L-BH<sub>4</sub> ([3'-<sup>2</sup>H<sub>1</sub>]-L-BH<sub>4</sub>) (5). If L-primapterin originates from L-BH<sub>4</sub>, deuterium labelling should also be present in L-primapterin isolated from the urine after loading, and it can also be determined whether the side-chain is from the same origin.

### Material and Methods

Loading of a 5 year old male primapterinuric patient T. S. was performed with a dose of 10 mg deuterated L-BH<sub>4</sub> per kilogram body weight. His urine was collected up to 12 hours after loading.

The purification and isolation of urinary L-biopterin and L-primapterin after loading was performed using several different chromatographic and filtration steps: After acidic oxidation of the urine with iodine the acidic solution (0.1 N HCl) was applied to a Dowex 1 × 4 column. The column was washed with 0.1 N HCl and fractions fluorescing blue were collected. Purines were separated from pterins by precipitation in aqueous ammonia (pH 8–9). The alkaline solution was applied to a Dowex 50 × 8 column where pterins and salts passed through. The collected blue fluorescent fractions were made alkaline (pH 8–9) and then applied on a Dowex 1 × 4 column. Pterins were eluted with 0.1 N HCOOH. The eluate was made alkaline with NH<sub>4</sub>OH and applied to a Dowex 1 × 4 column and the pterins were fractionated with a 0.1 N NH<sub>4</sub><sup>+</sup>COO<sup>-</sup> buffer, pH 7.8. The pterin solution was made alkaline with NH<sub>4</sub>OH (pH 8–9) and applied to a second Dowex 1 × 4 column. The column was washed with water, and the pterins were eluted with 0.1 N HCOOH. Excess acid was removed by evaporation, and the residue was diluted with water. The separation of the pterins was performed using a preparative reverse-phase HPLC column (6). After the first preparative HPLC-separation L-primapterin fractions still contained some L-biopterin. Therefore a second preparative HPLC was necessary.

<sup>1)</sup> Author to whom correspondence should be addressed.

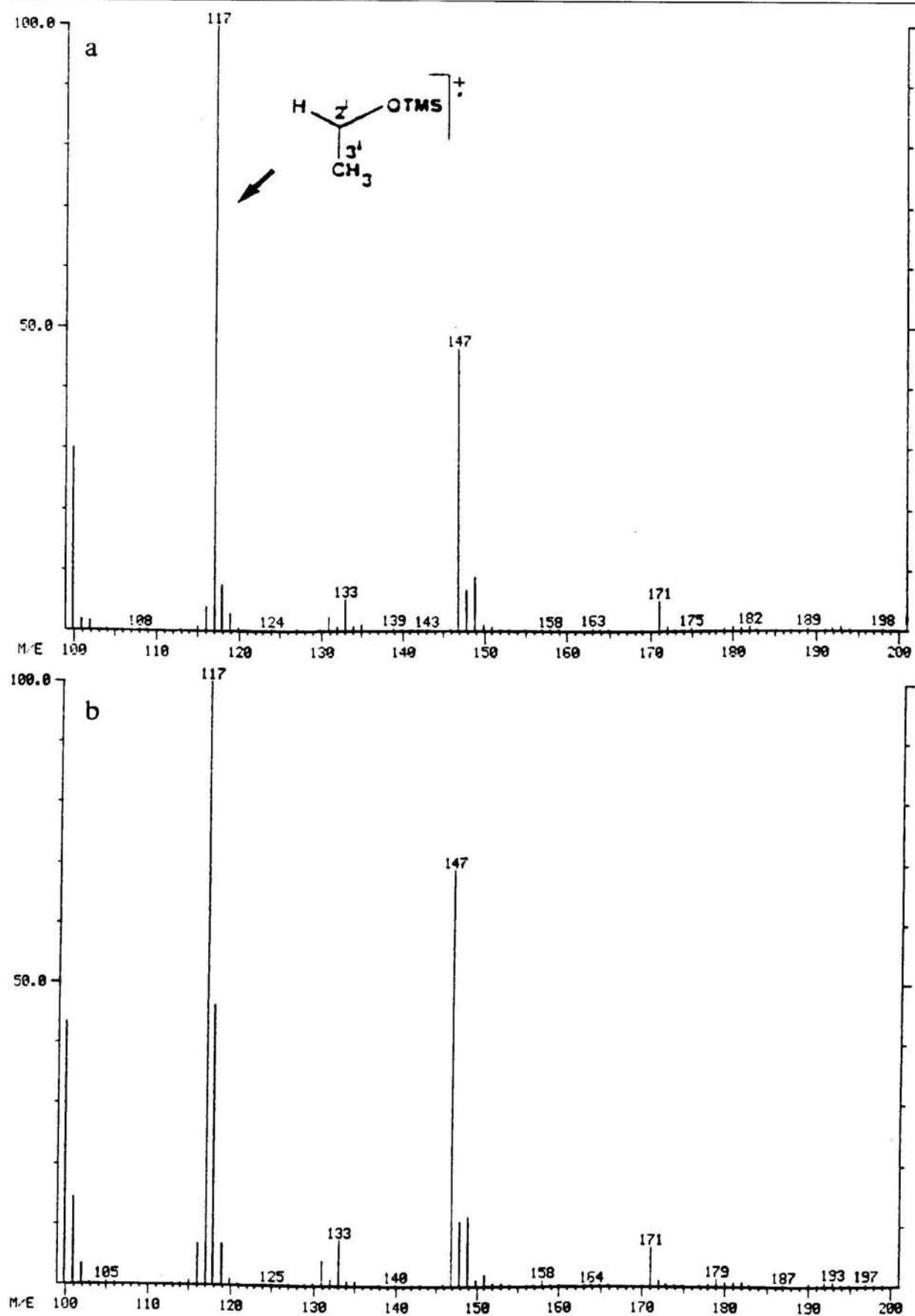


Figure 1. Low molecular fragments of L-primapterin. a) reference compound; b) isolated from urine after L-[3'-<sup>2</sup>H<sub>1</sub>]BH<sub>4</sub>-loading.

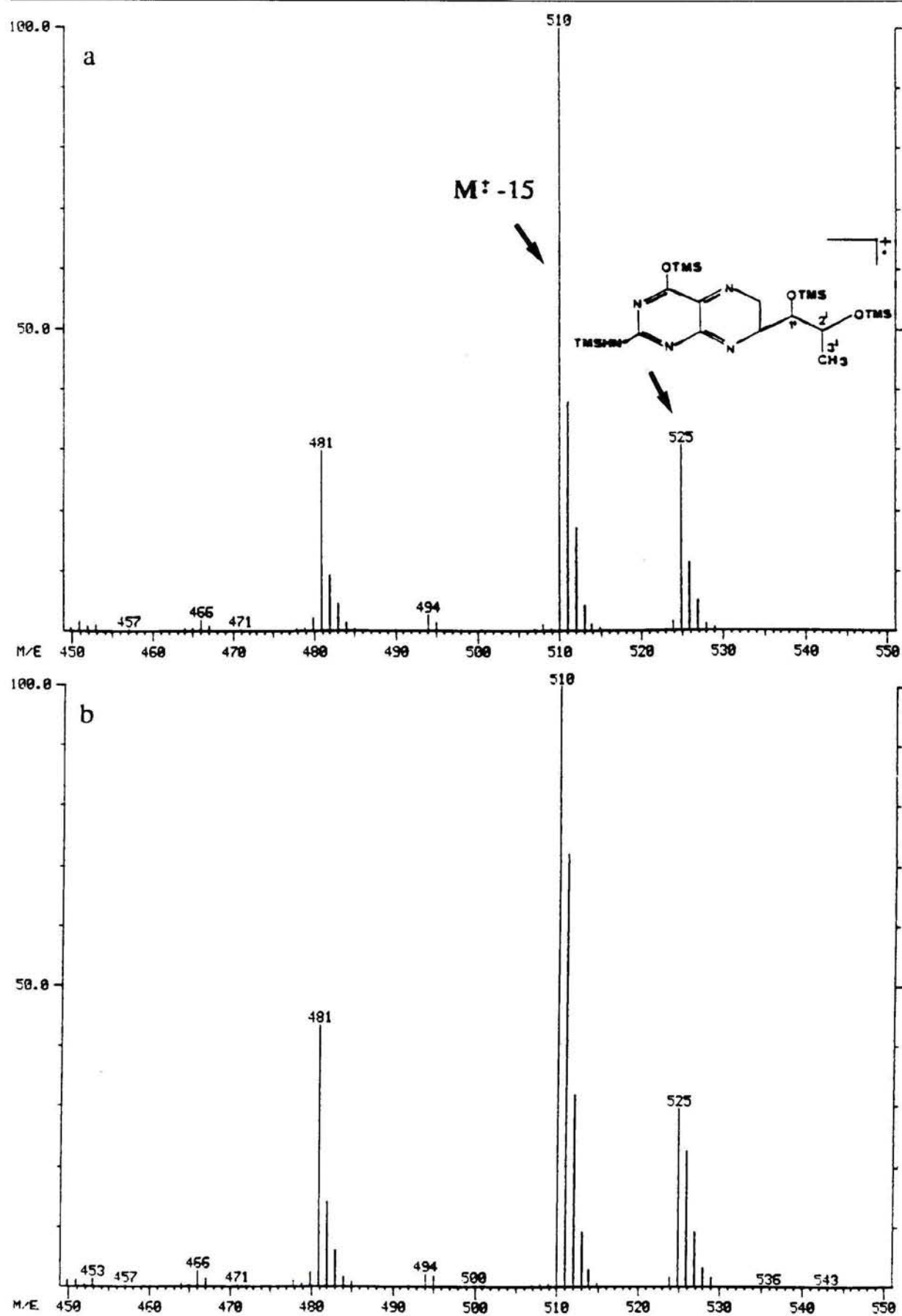


Figure 2. High molecular fragments of L-primapterin. a) reference compound; b) isolated from urine after L-[3'-<sup>2</sup>H]<sub>1</sub>]BH<sub>4</sub>-loading.

Using the above described method of purification and isolation 300  $\mu\text{g}$  of L-biopterin and 50  $\mu\text{g}$  of L-primapterin were isolated from 200 ml urine.

Gas-chromatography/mass-spectrometry was performed using the TMS-derivatives of L-biopterin and L-primapterin (7).

### Results and Discussion

We synthesized 3'-mono deuterated L-biopterin and analyzed it by GC/MS as a TMS-derivative. These data were compared with the MS data of unlabelled L-biopterin. Three ions were affected by a + 1 Da shift, i. e.  $m/z$  117 containing the C-2',3'-end of the side-chain,  $m/z$  510 originating from  $M^+ - 15$  often observed for TMS-derivatives and the molecular ion at  $m/z$  525.

L-biopterin as well as L-primapterin have the same electron impact-GC/MS fragmentation pattern, and differ only in their retention times on the GC column.

In the GC/MS spectrum of L-biopterin, isolated after the loading, a shift of + 1 Da was observed for the primary ions expected to exhibit deuterium incorporation, i. e.  $m/z$  117, 510, and 525, were shifted to  $m/z$  118, 511, and 526, respectively. Ion intensity values for  $m/z$  118, 511, and 526 were found to have increased by 40% for this deuterium labelled sample relative to the standard (data not shown).

The same is true for isolated L-primapterin after loading with deuterated L-biopterin. Again, all three relevant ions ( $m/z$  117, 510, and 525) are shifted

+ 1 Da (absolute intensity increase  $\sim 40\%$ ) indicative of deuterium incorporation (see Fig. 1 and 2).

This indicates clearly that the isolated L-primapterin originates from L-biopterin and, in addition, that the side-chain of L-primapterin derives from L-biopterin. With this experiment we can exclude an intermolecular reaction where the side-chain attaches to an already existing pterin ring nucleus.

### Acknowledgement

This project is financially supported by the Swiss National Science Foundation, project no. 31-28797.90.

### References

1. Curtius, H.-Ch., Kuster, T., Matasovic, A., Blau, N. & Dhondt, J.-L. (1988) *Biochem. Biophys. Res. Commun.* **153**, 715–721.
2. Curtius, H.-Ch., Matasovic, A., Schoedon, G., Kuster, T., Guibaud, P., Giudici, T. & Blau, N. (1990) *J. Biol. Chem.* **265**, 3923–3930.
3. Curtius, H.-Ch., Adler, C., Rebrin, I., Heizmann, C. & Ghisla, S. (1990) *Biochem. Biophys. Res. Commun.* **172**, 1060–1066.
4. Davis, M. D., Kaufman, S. & Milstien, S. (1991) *Proc. Natl. Acad. Sci.* **88**, 385–389.
5. Adler, C., Curtius, H.-Ch., S. & Viscontini, M. (1990) *Helv. Chim. Acta* **73**, 1058–1063.
6. Niederwieser, A., Staudenmann, W. & Wetzler, E. (1982) in: *Biochemical and Clinical Aspects of Pteridines* (Wachter, H., Curtius, H.-Ch. & Pfeleiderer, W., eds.) pp. 81–102, Walter de Gruyter, Berlin, New York.
7. Kuster, T. and Niederwieser, A. (1983) *J. Chromatogr.* **278**, 245–254.