105. Pterin Chemistry

Part 88[1])

A Simple Synthesis of 5-Deoxy-L-[5-$^2H_1$]Arabinose and L-[3′-$^2H_1$]Biopterin

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A simple synthesis of 5-deoxy-L-[5-$^2H_1$]arabinose was performed to obtain L-[3′-$^2H_1$]biopterin. The reduced form of this model substance is needed to investigate the pathway of 7-substituted pterins in patients with primapterinuria.

In the urine of a patient with atypical PKU, the 7-substituted pterins L-primapterin[3]) and D- or L-anapterin were detected parallel to L-biopterin and D-neopterin. The ratio of L-biopterin to L-primapterin was *ca.* 1:1, as measured by HPLC [2–4]. Because of a mild hyperphenylalaninemia, the patient was treated with 6β-tetrahydro-L-biopterin[4]) (BH_4). The excreted quantities of L-biopterin and L-primapterin increased, while the ratio of the two compounds remained unchanged [6]. It would be interesting to know what causes this change in the patient's metabolism. The results of the BH_4 treatment make it plausible that L-primapterin is formed from L-biopterin. To prove this hypothesis, labelled BH_4 must be administered to the patient. If the excreted L-primapterin is labelled, it can be assumed that it derives from L-biopterin.

L-Biopterin (**10**) is obtained by condensation of 2,5,6-triaminopyrimidin-4-ol with the acetylated hydrazone **7** of 5-deoxy-L-arabinose (see below). Therefore, we decided to label 5-deoxy-L-arabinose (**5**) with deuterium. This sugar had been synthesized manifold: on the one hand from L-rhamnose, where the CH_3(5) group already exists [7], and on the other hand from L-arabinose (**1**), where the CH_2(5)OH group has to be reduced [8].

The reduction at C(5) of **1** works best when its aldehyde group is protected as a diethyl dithioacetal ($\rightarrow$ **2**) and the CH_2(5)OH group is activated by tosylation ($\rightarrow$ **3**; *Scheme 1*). With $LiAlH_4$, the tosyloxy group of **3** can be reductively removed and the two ethylthio groups split off by $HgCl_2$ [8a]. For our new synthesis, we would neither use $LiAlH_4$, too dangerous in large quantities, nor $HgCl_2$ because of its toxicity. After several negative

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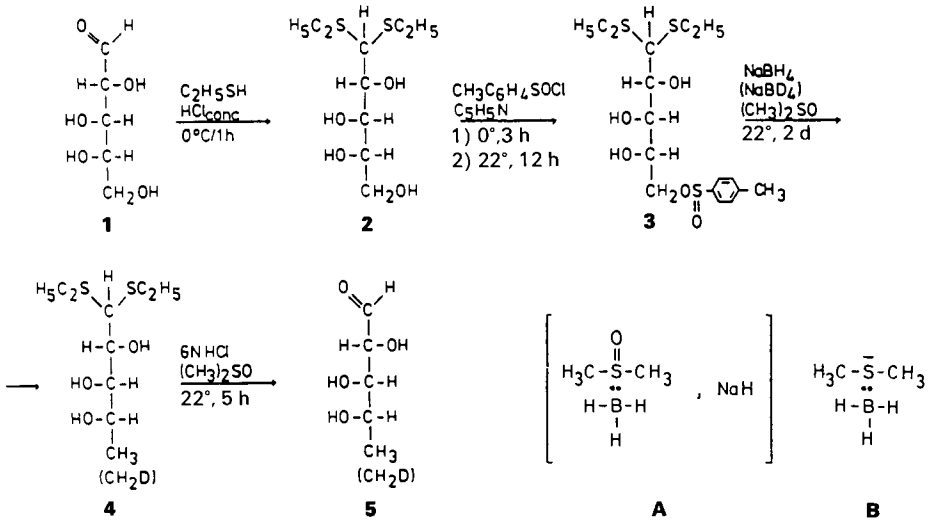
[3]) The configuration of this compound is given in *Helv. Chim. Acta* **1990**, *73*, 1064.

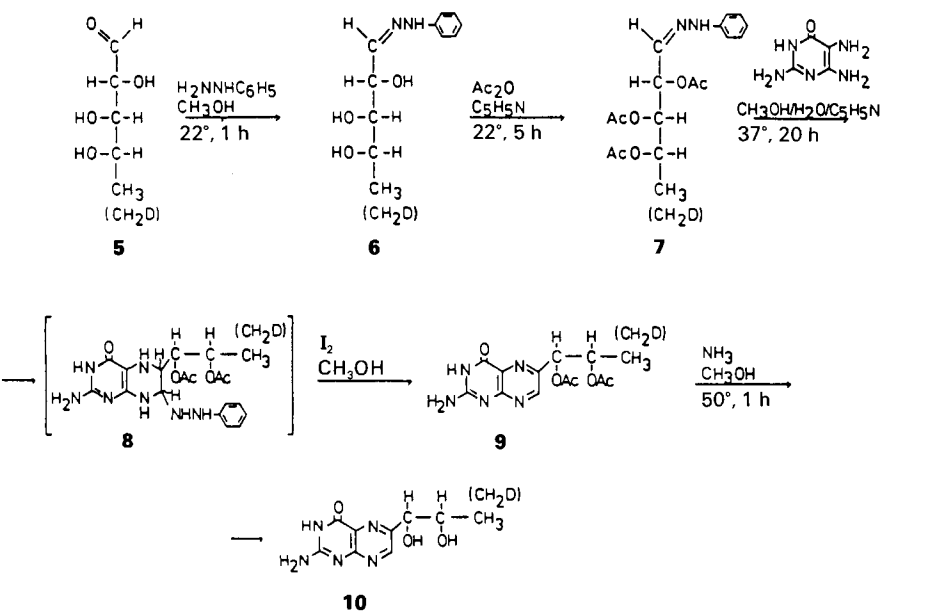
[4]) We use in this publication the designation rules for pterin derivatives proposed in [5].

attempts with different solvents, we discovered that the reduction of the tosyloxy group with $NaBH_4$ in H_2O-free dimethyl sulfoxide (DMSO) proceeds easily ($\rightarrow$ **4**) so that $NaBD_4$ can be used for labelling the 5-position ($\rightarrow$ [5-$^2H_1$]-**4**). It is possible that the sulfoxide can form a complex **A** with $NaBH_4$, in analogy to the known complex **B** which is a good reduction reagent [9].

Scheme 1



Scheme 2



The elimination of the two ethylthio groups from **4** (or [5-$^2H_1$]-**4**), impossible with HCl in several solvents (H_2O, EtOH, *etc.*), proceeds well, when DMSO is added to the aqueous HCl solution yielding the target compound **5** (and [5-$^2H_1$]-**5**, resp.) in 84% yield. It is known that dialkyl dithioacetals are in equilibrium with the corresponding aldehydes and thiols in the presence of HCl. Possibly, the free thiol (EtSH in the present case) reacts with DMSO (an oxidation reagent) forming a disulfide (Et-SS-Et).

L-Biopterine (**10**) and its labelled derivative [3′-$^2H_1$]-**10** were prepared from **5** and [5-$^2H_1$]-**5** *via* **6**–**9** and their labelled derivatives, respectively, according to the method of *Schircks et al.* [15] (see *Scheme 2* and *Exper. Part*).

By mass spectrometry, we have shown that the ^2H-label at C(5) or C(3′) does not exchange with a H-atom during the different reactions described in *Schemes 1* and *2*. The grade of deuteration remains a constant 80% for all steps.

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Experimental Part

1. *General.* M.p.: uncorrected; *Büchi-510* melting-point apparatus. TLC: silica gel plates $60F_{254}$ (*Merck*). Sugar detection with $NaIO_4$/benzidine test [10]. MS: *VG-16F* single-focusing magnetic-field instrument; volatile derivatives of all compounds were obtained with bis(trimethylsilyl)trifluoroacetamide [11].

2. L-*Arabinose Diethyl Dithioacetal* (**2**) was prepared according to [12]. M.p. 124° ([13]: 124–126°).

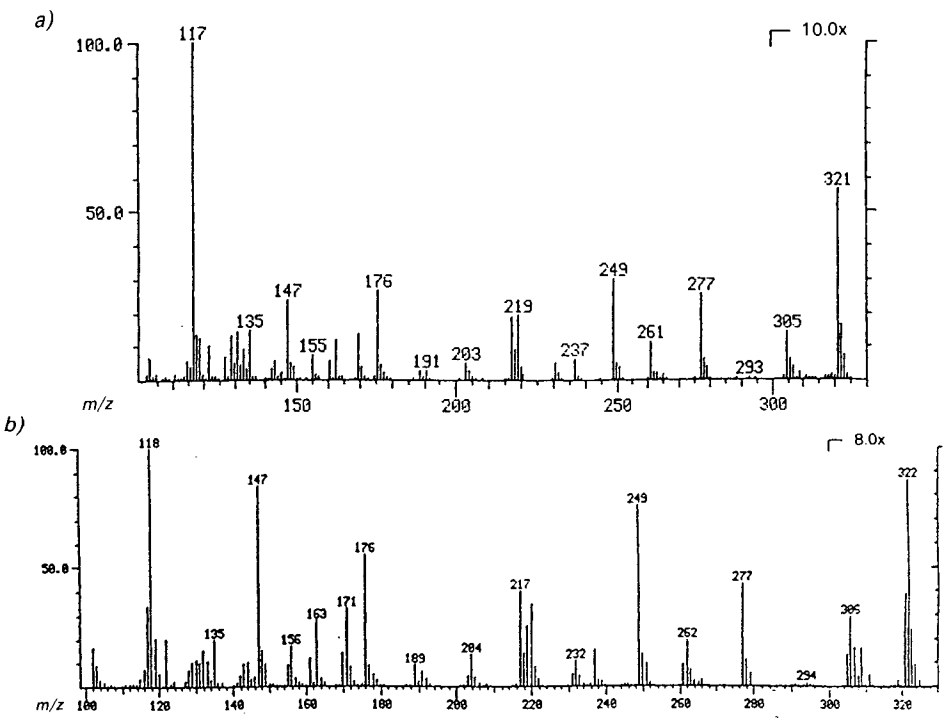

Fig. 1. *MS data of* a) *5-deoxy-*L*-arabinose diethyl dithioacetal* (**4**) *and* b) *5-deoxy-*L*-[5-$^2H_1$]arabinose diethyl dithioacetal* ([5-$^2H_1$]-**4**). **4**: most important peaks at *m/z* 117 and 321; [5-$^2H_1$]-**4**: most important peaks at *m/z* 118 and 322.

3. *5-O-Tosyl-*L*-arabinose Diethyl Dithioacetal* (**3**) was obtained according to [12a] and [14]. M.p. 67° ([14]: 68°).

4. *5-Deoxy-*L*-arabinose Diethyl Dithioacetal* (**4**). A mixture of 1.5 g (35 mmol) of $NaBH_4$ and 50 ml of DMSO was stirred to an almost clear soln. (*ca.* 1 h). Then, 10 g (24 mmol) of **3** in 50 ml of DMSO were added. The mixture was kept for 2 days at r.t. After evaporation (30°/0.1 Torr), the solid residue was cooled (ice-bath), and pieces of ice

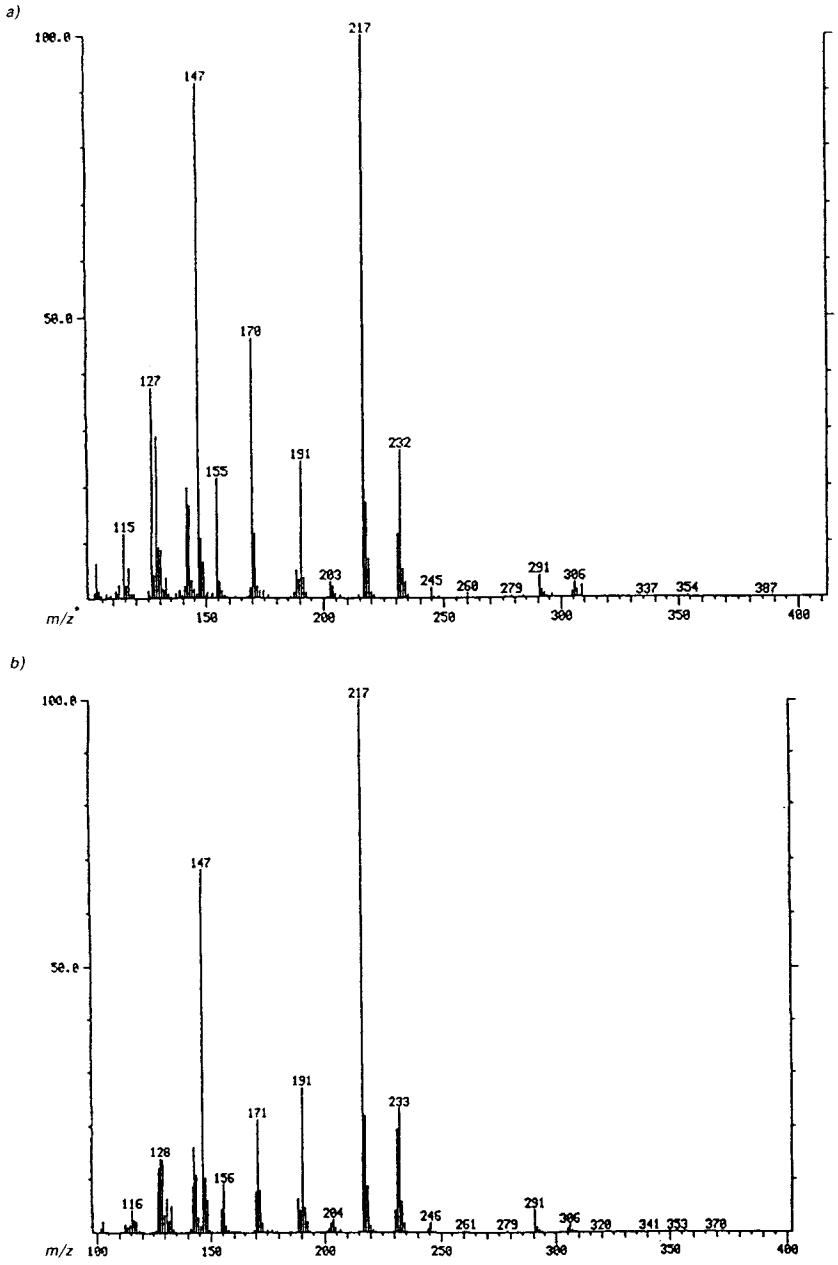

Fig. 2. *MS data of* a) *5-deoxy-*L*-arabinose* (**5**) *and* b) *5-deoxy-*L*-[5-$^2H_1$]arabinose* ([5-$^2H_1$]-**5**)

were added together with 5% AcOH until the H_2 development stopped. At this time, the pH must be 6.0–6.5. The remaining insoluble part was discarded and the soln. extracted with Et_2O. The Et_2O layer was dried (Na_2SO_4) and evaporated: 4 g (70%) of **4** as needles which were recrystallized from $CHCl_3$/pentane. M.p. 109° ([14]: 109°). MS: no $M^{+\cdot}$, 321 (fragment including C(2), C(3), C(4), C(5)), 117 ($[Me_3SiOCHMe]^+$); *Fig. 1a*.

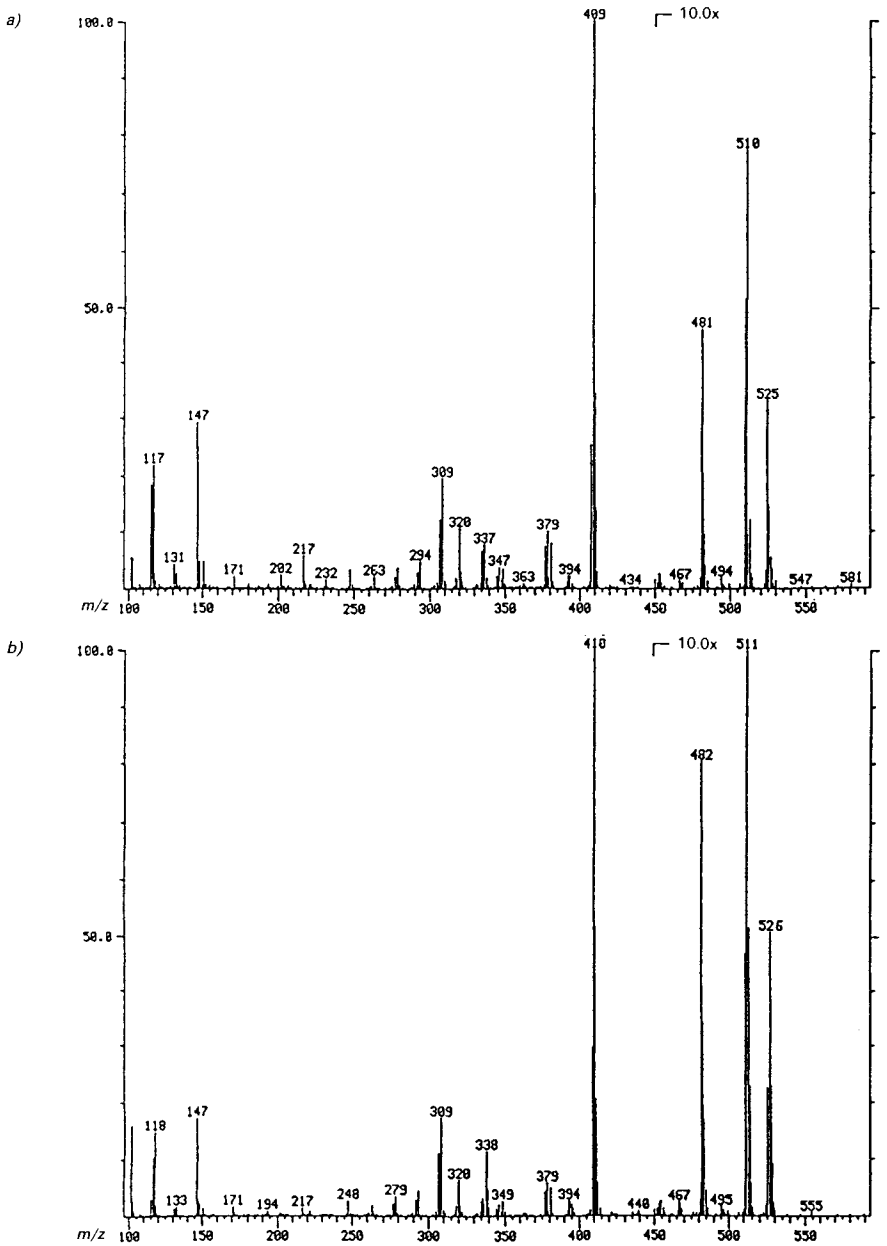

Fig. 3. *MS data of* a) L-*biopterin* (**10**) *and* b) L-*[3′-$^2H_1$]biopterin* ([3′-$^2H_1$]-**10**). **10**: most important peaks at *m/z* 117 and 525; [3′-$^2H_1$]-**10**: most important peaks at *m/z* 118 and 526.

5. *5-Deoxy-*L*-[5-$^2H_1$]arabinose Diethyl Dithioacetal* ([5-$^2H_1$]-**4**) was prepared as described in *Exper. 4* using $NaBD_4$. MS: no $M^{+\cdot}$, 322 (fragment including C(2), C(3), C(4), C(5)), 118 ($[Me_3SiOCH(CH_2{}^2H)]^{+}$); *Fig. 1b*.

6. *Deoxy-*L*-arabinose* (**5**). A mixture of **4** (3 g, 13 mmol) and 3.5 g (44 mmol) of DMSO in 32 ml of 6N HCl was stirred at r.t., until **4** was dissolved (4 h). After 1 h further stirring, a mixture of 2 homogeneous liquids was obtained. The aq. layer was separated, cooled with an ice-bath and slowly adjusted to pH 6.5 with 6N NaOH. The soln. was evaporated as far as possible. The precipitated NaCl was filtered off through a glass-sintered funnel (No. *3*) and washed with MeOH. The collected filtrate was concentrated. The newly formed NaCl was separated by filtration and washed with EtOH. Concentration, filtration, and washing (after the second time always with EtOH) were repeated until the formation of crystallized NaCl stopped. The yellow oily residue was dissolved in 20–30 ml of EtOH, and the soln. was brought drop by drop into Et_2O, thus, precipitating a white powder: 1.4 g (84%) of **5**. MS: complex because of presence of cyclic and open-chain form; no $M^{+\cdot}$, 115 (ev. fragment including C(4) and C(5), with double bond; *cf. m/z* 117 for **4**); *Fig. 2a*.

7. *5-Deoxy-*L*-[5-$^2H_1$]arabinose* ([5-$^2H_1$]-**5**). Same procedure as *Exper. 6*. MS: see **5**; no $M^{+\cdot}$, 116 (ev. fragment including C(4) and C(5), with double bond); *Fig. 2b*.

8. L-*Biopterin* (**10**) was prepared according to [15]. After oxidation of **8** with an I_2/MeOH soln. (3.6 g of I_2 in 50 ml of MeOH) and evaporation the residue (**9**) in 80 ml of MeOH was deacetylated by adding 100 ml of conc. NH_3 soln. (50°/1 h). After evaporation, the residue was dissolved in 200 ml of 0.2N NH_4OH and the insoluble part filtered off. In contrast to [15], the salts of this filtrate were eliminated by applying it at pH 9–10 to a *Dowex 1 × 4* column (50 × 100 mm), eluating the adsorbed pterins with 0.01N AcOH, evaporating, dissolving the residue in 0.2N NH_4OH, and applying the soln. to a *Dowex 1 × 8* column (50 × 379 mm) as described in [16]: 250 mg (13%) of **10**. MS: 525 ($M^{+\cdot}$ of tetrakis(trimethylsilyl)-substituted **10**), 117 (fragment including C(2′) and C(3′) of side chain; *cf. m/z* 117 for **4**); *Fig. 3a*.

9. L-*[3′-$^2H_1$]Biopterin* ([3′-$^2H_1$]-**10**). Same procedure as *Exper. 8*. MS: see **10**; 526 ($M^{+\cdot}$ of tetrakis-(trimethylsilyl)-substituted [3′-$^2H_1$]-**10**), 118 (fragment including C(2′) and C(3′) of side chain); *Fig. 3b*: many peaks 1 unit higher than in *Fig. 3a*, *i.e.* on fragmentation, the side chain remains attached to the pyrazine ring.

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