169. Pterinechemistry

Part 84[1])

A New, Regiospecific Synthesis of L-Biopterin[2])

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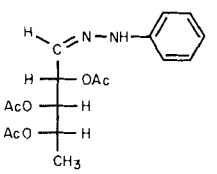
Pure L-biopterin was obtained in 42% yield by the condensation of 5-deoxy-L-arabinose-phenylhydrazone-triacetate with 4-hydroxy-2,5,6-triaminopyrimidine, followed by iodine oxidation of the formed tetrahydropterin derivative to 1',2'-*O*-diacetyl-L-biopterin. Deacetylation was carried out with NH_4OH.

In [2], we reported on a new, regiospecific synthesis of L-biopterin (**1**; *cf.* the *Scheme*), whose history has been published in [3]. This new synthesis has been developed according to the fact that the condensation of 4-hydroxy-2,5,6-triaminopyrimidine (**2**) with 5-deoxy-L-arabinose-phenylhydrazone (**3**) yields as an intermediate a regiospecific 6-(1',2'-dihydroxypropyl)-5,6,7,8-tetrahydro-L-biopterin derivative (**7**), [4] and that protection of the two OH groups in the side chain will prevent its cleavage during the oxidation of the tetrahydro-L-biopterin derivative to L-biopterin [5].

The most difficult part of the synthesis is the protection of the OH groups of the 5-deoxy-L-arabinose (**4**). Since the direct acetylation of **4** did not give the desired product,



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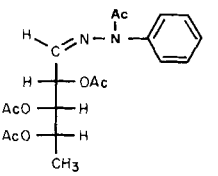

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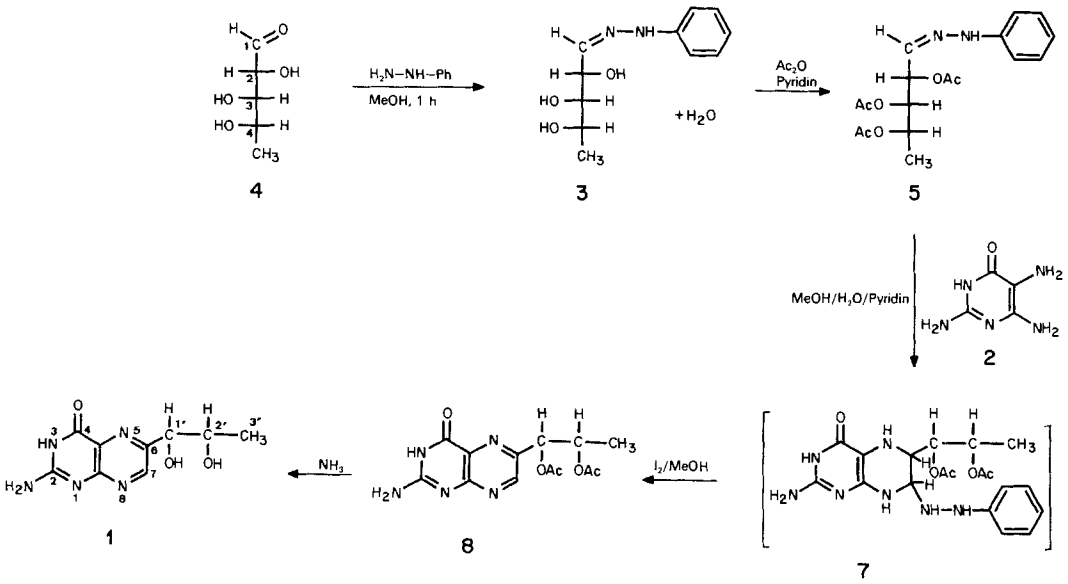
we tried the acetylation of the starting material *i.e.* 5-deoxy-L-arabinosephenylhydrazone (**3**). Depending on the acylation conditions, the compounds **5** and **6** could be obtained. The structure determinations were based on ^{1}H-NMR spectra. Despite the fact that from theoretical reasons neither **5** nor **6** would undergo a classical *Amadori* rearrangement, which seemed to us a necessary step before the condensation with **2**, preliminary attempts, however, showed that the triacetyl compound **5** reacted easily with **2**, involving probably an intramolecular acetyl migration from C(2), while the tetraacetyl compound **6** did not react. This result allowed us to develop the following synthesis of L-biopterin (*Scheme*).

[1]) Part 83: [1].

[2]) Part of the Ph.D. thesis of *B. S.*, Universität Zürich, 1978.

Scheme



5-Deoxy-L-arabinose (**4**) obtained by the method of *Taylor* and *Jacobi* [6] was first transformed into the phenylhydrazone **3** and then acetylated to **5** with Ac_2O in pyridine. The triacetate **5** was then reacted with **2** in MeOH to form a 5,6,7,8-tetrahydropterin derivative **7** which could not be isolated, and to which we assigned tentatively the hypothetical structure **7**. This intermediate was then oxidized with I_2 to form 1′,2′-*O*-diacetyl-L-biopterin (**8**), which could be directly isolated. Cleavage of the Ac groups with NH_4OH led to pure L-biopterin.

The synthesis was very convenient, because it could be carried out without isolation of the intermediate products in a one-pot process. It was thus possible to improve the yields (40–60%), depending on the reaction conditions.

We are indebted to the following persons for their help: Mrs. *H. Schiegg* for her excellent technical assitance during the development of the L-biopterin synthesis, Mr. *K. Bachmann* (Laboratory of Prof. *W. von Philipsborn*) for ^{13}C- and ^{1}H-NMR spectra and Mr. *H. Frohofer* for elemental analysis.

Experimental Part

1. *General.* All reactions were performed under orange light. ^{1}H-NMR spectra were recorded on a *Varian-HA-100* instrument (TMS as internal standard) and ^{13}C-NMR spectra on a *Varian-LX-100-15* spectrometer.

2. *5-Deoxy-L-arabinose* (**4**). The compound was prepared according to [6].

3. *5-Deoxy-L-arabinose-phenylhydrazone* (**3**). To the purified **4** (300 mg, 1.97 mmol) dissolved in MeOH (60 ml) was added under N_2 pure phenylhydrazine (240 mg, 2.22 mmol), followed by one drop of glacial AcOH. After 1 h at r.t., the yellow soln. was evaporated under vacuum (35–40°/12 Torr). The viscous residue was washed with Et_2O (2 × 15 ml) which was discarded. The remaining substance was dissolved in AcOEt (10 ml), the obtained soln. was washed twice with H_2O (*ca.* 10 ml each time), the org. layer was dried (Na_2SO_4) and evaporated under

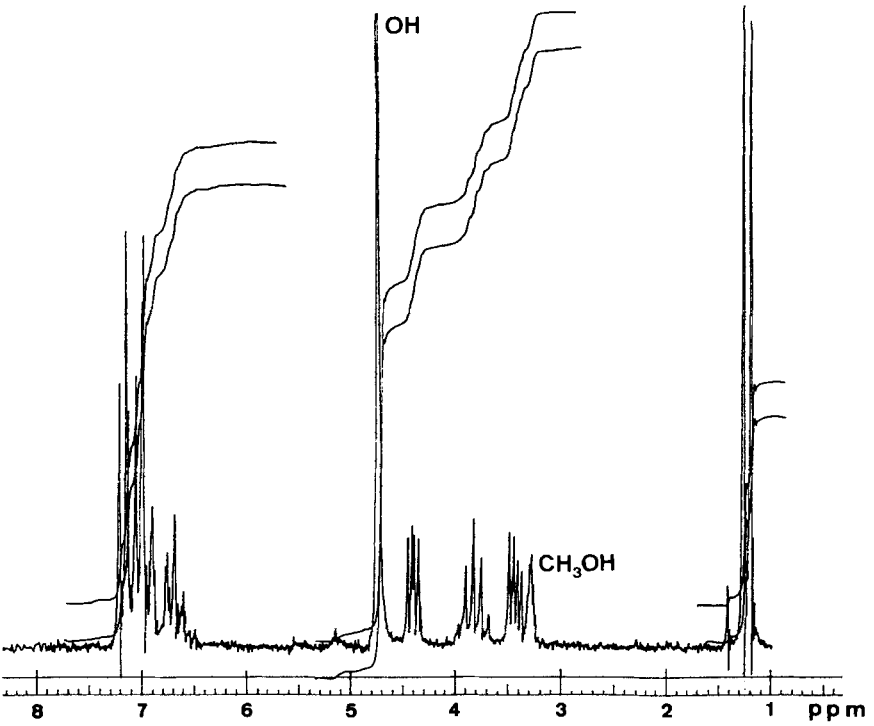


Fig. 1. *^{1}H-NMR spectrum of* **3** *in CD_3OD*

vacuum. The residue crystallized after several hours: 125 mg (71%) **3**. ^{1}H-NMR (CD_3OD; *Fig. 1*): 7.35–6.55 (*m*, 5 arom. H); 7.19 (*d*, *J* = 6.0, H–C(1)); 4.45 (*q*, *J* = 3.6, H–C(2)); 3.87 (*m*, *J* = 6.4, 6.2, H–C(4)); 3.48 (*q*, H–C(3)); 1.28 (*d*, *J* = 6.2, CH_3–C(4)). Anal. calc. for $C_{11}H_{16}N_2O_3$ (224.27): C 58.91, H 7.19, N 12.49; found: C 59.37, H 7.67, N 11.16.

4. *5-Deoxy-2,3,4-*O*-triacetyl-*L*-arabinose-phenylhydrazone* (**5**). To the crystallized **3** (100 mg, 0.45 mmol) dissolved in Ac_2O (1 ml) was added, under N_2, pyridine (1 ml). After 5 h at 22°, the soln. was evaporated under vacuum and the residue dried (40°/0.01 Torr, 15 h): 156 mg (100%) **5**. ^{1}H-NMR ($CDCl_3$; *Fig. 2*): 7.90 (*s*, –NH–N=); 7.55–6.80 (*m*, 5 arom. H); 6.93 (*d*, H–C(1)); 5.74 (*m*, H–C(2)); 5.52 (*m*, H–C(3)); 5.17 (*m*, H–C(4)); 2.15 and 2.09 (2*s* and 1*s*, 3 CH_3CO)); 1.36 (*d*, *J* = 6.2, CH_3–C(4)).

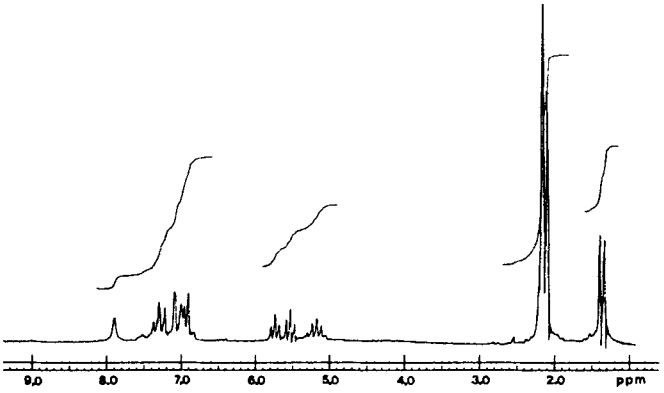


Fig. 2. *^{1}H-NMR spectrum of* **5** *in $CDCl_3$*

5. *Tetraacetyl-5-deoxy-*L*-arabinose-phenylhydrazone* (tentatively **6**). To Ac_2O (4 ml) was added recrystallized **3** (100 mg, 0.45 mmol). The soln. was warmed 2 h at 80°, then evaporated under vacuum and the residue purified by column chromatography on silica gel (benzene/MeOH 19:1). The fraction containing **6** was concentrated under vacuum and dried (40°/0.01 Torr, 15 h): 128 mg (73%) **6**. ^{1}H-NMR ($CDCl_3$): 7.60–6.67 (*m*, 5 arom. H); 6.33 (*d*, *J* = 4.5, H–C(1)); 5.62 (*m*, *J* = 4.5, H–C(2)); 5.27 (*q*, *J* = 6.4, H–C(3)); 5.05 (*m*, *J* = 6.0, H–C(4)); 2.43, 2.04, 2.00, 1.98 (4*s*, 4 CH_3CO); 1.20 (*d*, *J* = 6.2, CH_3–C(4)).

6. *1',2'-*O*-Diacetyl-*L*-biopterin* (**8**) [7]. To a soln. of **5** (300 mg, 0.85 mmol) in MeOH (30 ml) was added, under N_2, pyridine (1.5 ml), $Na_2S_2O_4$ (30 mg) dissolved in H_2O (12 ml) and 4-hydroxy-2,5,6-triaminopyrimidine (**2**; 200 mg, 0.95 mmol) dissolved in H_2O (12 ml). After 20 h (35–40°), a soln. of I_2 (0.51 g, 2.01 mmol) dissolved in MeOH (20 ml, 22°) was added to oxidize the tetrahydropterine derivative **7**. While stirring, a brown substance precipitated. After 30 min the presence of excess I_2 was determined with starch-paper. If no excess was present, a small quantity of I_2 was added in order to complete the oxidation of **7**. After reduction of excess I_2 with $Na_2S_2O_4$, the obtained soln. was evaporated under vacuum and dried (12 Torr/40°, 15 h). The dark brown residue was taken of in H_2O (20 ml, 22°), the crude **8** filtered, washed with a small quantity of cold H_2O, cold MeOH, Et_2O, and dried (40°/0.01 Torr, 15 h): 72 mg (26%) crude **8**. TLC (silica gel; MeOH/benzene 20:80) showed a mixture consisting of a small quantity of pterin, mono-acetyl-L-biopterin, and diacetyl-L-biopterin as main product (R_f (diacetate) 0.35; R_f (monoacetate) 0.15; R_f (pterin) and R_f (biopterin) 0). For the purification of **8**[3]), 50 mg of the crude mixture dissolved in the minimum amount of AcOEt was put on a silica-gel column (20 × 4 cm) and was first eluted with benzene followed by AcOEt to remove the impurities, and then with MeOH/benzene 1:19. The fractions were checked by silica-gel TLC. Those containing **8** were evaporated to dryness. The residue, which contained some silica gel, was dissolved in hot H_2O, filtered, and the filtrate evaporated to dryness.

Recrystallisation from hot H_2O was not done because much monoacetyl-biopterin is formed during this operation. ^{1}H-NMR ((D$_6$)DMSO): 8.65 (*s*, H–C(7)); 7.0 (br. *s*, H_2N(2)); 5.85 (*d*, *J* = 4.6, H–C(1')); 5,25 (*m*, H–C(2')); 2.15, 1.90 (2*s*, 2 CH_3CO); 1.15 (*d*, *J* = 5.6, CH_3–C(2')). Anal. calc. for $C_{13}H_{15}N_5O_5$ (321.29): C 48.60, H 4.70, N 21.80; found: C 47.77, H 4.55, N 21.77.

7. L-*Biopterin* (**1**). A soln. of **8** (100 mg, 0.36 mmol) in H_2O (10 ml) and conc. NH_4OH (10 ml) was warmed 1 h to 50°. Then the soln. was evaporated under vacuum, the residue dissolved in 1% NH_4OH (80 ml) and the soln. passed through a *Dowex 1* × *8* column (15 × 4.5 cm), where the biopterin was adsorbed at the top. The chromatogram was developed with a 0.15 N ammonium-formate buffer solution, pH 9. The first fraction with blue fluorescence contained L-biopterin (control with cellulose TLC, 3% NH_4Cl in H_2O). It was evaporated to 100 ml under vacuum. After 15 h at 3–5°, the precipitated L-biopterin was filtered, dissolved in hot H_2O, treated with charcoal, and recrystallized. Filtration, washing with EtOH, Et_2O, and drying (40°/0.01 Torr, 15 h) gave 57 mg (67%) pure **1**. Solubility: 19 mg in 100 ml H_2O (22°). $[\alpha]_D^{20} = -66 \pm 2°$ (*c* = 0.2, 0.1 N HCl). ^{1}H-NMR (3 N DCl; *Fig.* in [2]): 9.37 (*s*, H–C(7)); 5.34 (*d*, *J* = 4.8, H–C(1')); 4.66 (*m*, *J* = 6.2, H–C(2')); 1.62 (*d*, *J* = 6.0, CH_3–C(2')). ^{13}C-NMR (3 N NaOD, *Fig.* in [2]): 173.60 (C(4)); 164.08 (C(2)); 155.70 (C(8a)); 153.56 (C(6)); 148.35 (C(7)); 127.97 (C(4a)); 78.76 (C(1')); 71.84 (C(2')); 19.59 (C(3')). Anal. calc. for $C_9H_{11}N_5O_3$ (237.22): C 45.57, H 4.67, N 29.53; found: C 45.19, H 4.96, N 29.48.

8. *One-pot Synthesis of* **1**. A suspension of 1,1-diethylsulfonyl-L-rhamnose (14 g, 42.1 mmol) [6] in H_2O (120 ml) was treated slowly with 4 N NH_4OH while stirring until the pH of the solution reached 9–10. After standing 24 h (22°), with stirring from time to time, the precipitate of diethylsulfonylmethane was filtered off and the filtrate evaporated to dryness (35–40°/12 Torr). The residue was dissolved in MeOH (80 ml). After adding pure phenylhydrazine (5 ml, 46 mmol), the soln. was kept at r.t. for 1 h and then dried under vacuum. The residue was washed two or three times with Et_2O (*ca.* 50 ml each time) and dried. The residue was dissolved in pyridine (35 ml) and the soln. was cooled to 0°. Then Ac_2O (35 ml) was added slowly, always under cooling. The obtained soln. was allowed to stand for 10 min in an ice-bath and was then kept at r.t. for 5 h. After addition of MeOH (200 ml) and another 15 h, at r.t., $Na_2S_2O_4$ (1 g) and $NaCH_3COO \cdot 3H_2O$ (12.5 g) dissolved in H_2O (300 ml), and 4-hydroxy-2,5,6-triaminopyrimidine dihydrochloride (10.3 g, 48.1 mmol) dissolved in H_2O (500 ml) were added successively to MeOH/pyridine. The whole mixture was stirred under N_2 gently at 35–40° for 20 h to give a homogeneous, reddish brown solution. The tetrahydro-condensation product **7** was oxidized by adding I_2 (25 g, 98.7 mmol) dissolved in MeOH (300 ml). Excess I_2, if present, was removed with sodium thiosulfate. If no excess was present, a small quantity of I_2 soln. was added in order to complete the oxidation of **7**. During the oxidation, a fine brown precipitate was formed. Then the soln. was evaporated under vacuum (35–40°) to 100 ml. MeOH (150 ml) and conc.

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[3]) The purification of **8** was carried out by Dr. *Abhoy N. Ganguly*, whom we thank for his cooperation.

NH_4OH (200 ml) were added to this remaining soln. which was warmed to 50° for 1 h. After evaporation to dryness under vacuum, the crude, brown colored biopterin was washed with cold H_2O (100–200 ml), cold MeOH (100–200 ml), and dried. The substance was dissolved in 1% NH_4OH (2.2 l) and passed through a *Dowex 1* × *8* column (30 × 7 cm), where the biopterin was adsorbed at the top. Elution and purification of **1** were carried out as described in *7*: 4.2 g (42%, calculated from diethylsulfonyl-L-rhamnose [6]).

REFERENCES

[1] F. Stierli, J. H. Bieri, M. Viscontini, *Chimia* **1984**, *38*, 429.
[2] B. Schircks, J. H. Bieri, M. Viscontini, *Helv. Chim. Acta* **1977**, *60*, 211.
[3] M. Viscontini, in 'Biochemical and Clinical Aspects of Pteridines', Ed. W. Pfleiderer, H. Wachter, and H.-C. Curtius, Walter de Gruyter, Berlin–New York, 1984, Vol. 3, pp. 19–33.
[4] M. Viscontini, R. Provenzale, *Helv. Chim. Acta* **1969**, *52*, 1225; M. Viscontini, R. Provenzale, W. Frei, *ibid.* **1972**, *55*, 570.
[5] M. Viscontini, W. Frei, *Helv. Chim. Acta* **1972**, *55*, 574.
[6] E. C. Taylor, P. A. Jacobi, *J. Am. Chem. Soc.* **1974**, *96*, 6781; *ibid.* **1976**, *98*, 2301.
[7] B. Schircks, in 'Neue regiospezifische Synthese von L-Biopterin und von dessen Derivaten', Dissertation, Universität Zürich, 1978; M. Kappel, R. Mengel, W. Pfeiderer, *Liebigs Ann. Chem.* **1984**, 1815.