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The Convenient Syntheses of Biopterin and Its Three Opitical Isomers

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Synopsis. Simple syntheses of biopterin (**4a**) and its three optical isomers (**4b, c, d**) are described. The condensation of 2,4,5-triaminopyrimidin-6(1*H*)-one (**1**) with each of the 5-deoxypentose phenylhydrazones (**2a, b, c, d**), followed by oxidation with $K_3[Fe(CN)_6]$ and H_2O_2 in the presence of KI, gives biopterin (**4a**) and the isomers (**4b, c, d**) respectively.

Biopterin [2-amino-6-(L-*erythro*-1,2-dihydroxypropyl)-pteridin-4(3*H*)-one] (**4a**), first isolated from human urine,[1,2] has attracted considerable interest because of its biological activities: biopterin is a growth factor for *Crithidia fasciculata*[1,2] and its tetrahydro derivative has been found to be a potent cofactor for phenylalanine,[3] tyrosine,[4] and tryptophan hydroxylases.[5] Because of this importance, much effort has been expended on the synthesis of biopterin (**4a**)[2,6,7,8] and its optical isomers (**4b**, **c**, **d**).[6c] The resulting methods, however, all suffer from difficulties, *e.g.*, in separating the biopterin (**4a**) from its isomer, isobiopterin (**5a**),[2,6,8] or in making appropriate starting materials.[7,8b,c] Accordingly, we sought a more general simple synthesis.

In 1970, Viscontini *et al.*[9] synthesized D-*erythro*-neopterin (**4e**) by the condensation of 2,4,5-triaminopyrimidin-6(1*H*)-one (**1**) with D-arabinose phenylhydrazone (**2e**). In this reaction, a tetrahydropteridine (**3e**) was formed which, on aerial oxidation at pH 7, gave neopterin (**4e**) unaccompanied by the 7-isomer (**5e**).

When we applied this method to the synthesis of biopterin (**4a**), though, using 5-deoxy-L-arabinose phenylhydrazone (**2a**) instead of **2e**, we obtained no biopterin. The only isolable product was the 6-unsubstituted pteridine (**4f**), apparently formed by the loss of the 6-dihydroxypropyl group during the oxidation step. A similar reaction was also observed by Viscontini and Provenzale.[8a] We investigated further the conditions of oxidation and found that the use of a mixture of $K_3[Fe(CN)_6]$ and H_2O_2 in the presence of KI in an acidic solution gave biopterin as the main product.

We synthesized 5-deoxy-L-arabinose by the method Hough and Taylor,[10] involving the oxidative degradation of L-rhamnose diethyl dithioacetal. 5-Deoxy-D-arabinose and 5-deoxy-L (and D)-xylose were made from the corresponding pentose by the procedures of Zinner, Wessely, and Kristen.[11] Heating 5-deoxy-L-arabinose phenylhydrazone (**2a**) with 2,4,5-triaminopyrimidin-6(1*H*)-one (**1**) in 50% aqueous methanol under nitrogen gave a compound unstable to oxidation, probably the tetrahydropteridine (**3a**), by analogy with **3e**.[9] When the intermediate was oxidized by air in a neutral or weakly alkaline solution, only the 6-unsubstituted pteridine (**4f**) was formed. However, when the same oxidation was carried out under acidic conditions, a small amount of biopterin (**4a**) was formed, together with **4f**. Eventually, we found that the intermediate (**3a**) was oxidized effectively to biopterin (**4a**) in an acidic solution using a mixture of $K_3[Fe(CN)_6]$ and H_2O_2 in the presence of a little KI. A small amount (*ca.* 20%) of the unsubstituted pteridine (**4f**) was formed, but no 7-isomer (**5a**) was detected. The separation of biopterin from **4f** and several other minor components was achieved easily by chromatography, first on a Florisil column using 2M-hydrochloric acid as the developer, then on another Florisil column by gradient elution using 0—1% ammonia,[12] and finally on a P-Cellulose column[6b,c] eluted by water. The structure of the product was confirmed by its UV spectra.

The three optical isomers (**4b**, **c**, **d**) were made similarly by the condensation of the pyrimidine (**1**) with the appropriate phenylhydrazone (**2b**, **c**, or **d**).

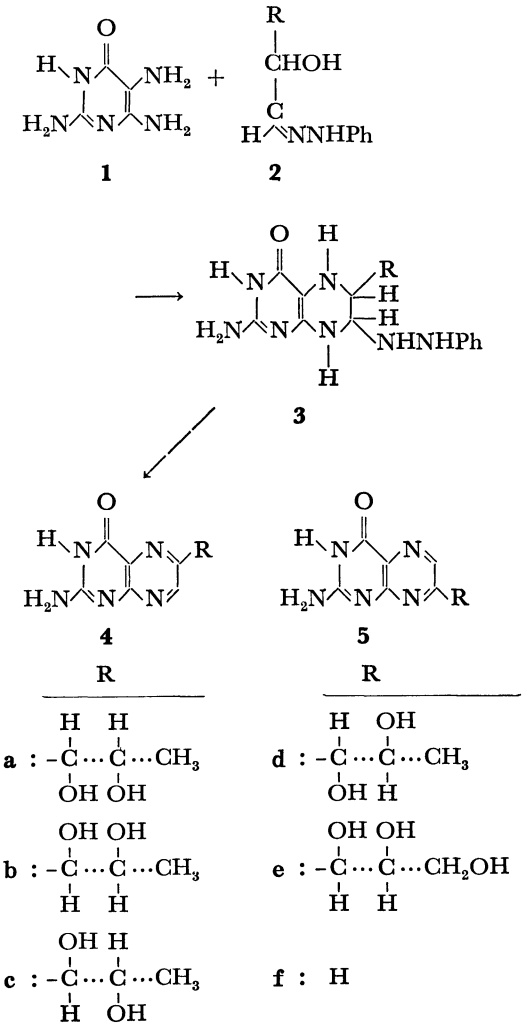

Table 1. pK_a Values and UV spectra of biopterin and its optical isomers

	pK_a	pH Ionic species[a]	λ_{max} (log ε)[b]			
4a	2.23±0.02	0.0 +	212 (4.21),	248 (4.09),	321 (3.94)	
	7.89±0.01	5.0 ○	*223 (4.07)*,	235.5(4.10),	274.5(4.18),	345 (3.82)
		10.0 −	221.5(3.92),	254.5(4.38),	364 (3.84)	
4d	2.23±0.02	0.0 +	212 (4.20),	248 (4.07),	321 (3.93)	
	7.90±0.01	5.0 ○	*223 (4.09)*,	235.5(4.10),	274 (4.17),	345 (3.82)
		10.0 −	224 (3.97),	255 (4.37),	364 (3.89)	
4c	2.24±0.02	0.0 +	213 (4.18),	248 (4.07),	322.5(3.93)	
	7.87±0.02	5.0 ○	*222 (4.05)*,	236 (4.09),	274 (4.17),	346 (3.81)
		10.0 −	224 (3.94),	254 (4.37),	364 (3.89)	
4d	2.20±0.02	0.0 +	213 (4.19),	248 (4.07),	321.5(3.94)	
	7.92±0.02	5.0 ○	223 (4.04),	236 (4.07),	274.5(4.15),	345.5(3.78)
		10.0 −	223 (3.94),	255.5(4.38),	364 (3.89)	

a) +; cation, ○; neutal molecule, −; anion. b) Shoulders are in italics.

Experimental

The analyses were done by the Analytical Section, Meijo University, Nagoya; the UV spectra were measured with a Shimadzu model UV-210 spectrophotometer.

L-Erythro-*biopterin (4a)*. Phenylhydrazine (3.6 g) and one drop of acetic acid were added to 5-deoxy-L-arabinose[10] (4.0 g) in ethanol (5 ml). After stirring at 20 °C for 30 min, the mixture was diluted with ether (5 ml) and chilled to give fine needles (3.6 g) of the phenylhydrazone (**2a**), which were then collected by filtration and washed with ether. A solution of this product (3.6 g) and 2,4,5-triaminopyrimidin-6(1H)-one dihydrochloride (3.0 g) in 50% aqueous methanol (400 ml) was stirred under nitrogen at 20 °C for 2 hr, and then under reflux for 4 hr. After cooling to *ca.* 20 °C, potassium ferricyanide (5.0 g), potassium iodie (0.10 g), and hydrogen peroxide (35%, 5 ml) were added quickly to the solution, and the mixture was stirred by introducing air at 20—25 °C for 12 hr. The mixture was concentrated under reduced pressure to *ca.* 50 ml and then chilled. The dark solid was collected by filtration, washed with a little cold water, and then extracted with 1% ammonia (2×100 ml). The extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 2M hydrochloric acid (100 ml) and fractionated on a Florisil column[12] (4×40 cm), using 2M hydrochloric acid as the developer. Biopterin was well separated from several minor by-products, including **4f**, during this procedure. The fractions containing biopterin were evaporated to dryness under reduced pressure, and the residue was extracted again with 1% ammonia (200 ml); a considerable amount of an inorganic material remained undissolved. The extract was evaporated to dryness and chromatographed on a Florisil column (4×30 cm), and then developed by gradient elution with water (1.0 l) and 1% ammonia (1.0 l). The biopterin fractions were finally purified on P-Cellulose[11] (acid form; 3×30 cm; eluted with water) to remove a small amount of colored impurities. The evaporation of the final eluate gave a solid, which was dissolved in 1% ammonia (50 ml) and then added drop by drop into vigorously stirred boiling formic acid (1M, 50 ml). Chilling gave almost colorless needles (270 mg) of L-*erythro*-biopterin (**4a**); mp>300 °C (Found: C, 43.9; H, 4.5; N, 28.3%. Calcd for $C_9H_{11}N_5O_3 \cdot 1/2H_2O$: C, 43.9; H, 4.9; N, 28.4%).

Synthesis of the Optical Isomers (4b, c, d). 5-Deoxy-L-xylose (2.5 g), phenylhydrazine (2.0 g), and 6M-HCl (5 drops) were stirred in methanol (30 ml) at 40—45 °C for 15 min. Without isolating the phenylhydrazone (**2c**), the solution was treated with the pyrimidine dihydrochloride (5.0 g) as above. Chromatographic purification as before afforded colorless needles (495 mg) of L-*threo*-biopterin (**4c**);[6c] mp>300 °C (Found: C, 44.1; H, 4.5; N, 28.0%. Calcd for $C_9H_{11}N_5O_3 \cdot 1/2H_2O$: C, 43.9; H, 4.9; N, 28.4%).

Replacing the 5-deoxy-L-xylose in the foregoing condensation by 5-deoxy-D-arabinose and 5-deoxy-D-xylose gave 205 mg of D-*eryhtro*-biopterin(**4b**)[6c] (mp>300 °C) (Found: C, 42.5; H, 4.6; N, 27.9%. Calcd for $C_9H_{11}N_5O_3 \cdot H_2O$: C, 42.35; H, 5.1; N, 27.4%) and 250 mg of D-*threo*-biopterin (**4d**)[6c] (mp>300 °C) (Found: C, 43.75; H, 4.6; N, 28.4%. Calcd for $C_9H_{11}N_5O_3 \cdot 1/2H_2O$: C, 43.9; H, 4.9; N, 28.4%) respectively.

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