BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 3564—3566 (1972)

The Synthesis of 3′-Phosphate and 2′,3′-Cyclic Phosphate of 2-Amino-4-hydroxy-6-(1′,2′,3′-trihydroxypropyl)pteridine

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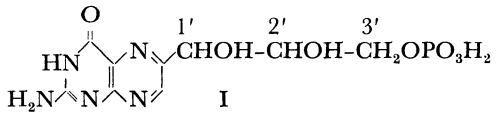
(Received February 9, 1972)

2-Amino-4-hydroxy-6-(D-*erythro*-1′,2′,3′-trihydroxypropyl)pteridine 3′-phosphate and 2′,3′-cyclic phosphate, important intermediates of pteridine biosynthesis, were prepared by simple methods. D-*erythro*-, L-*erythro*-, D-*threo*-, and L-*threo*-isomers of 2-amino-4-hydroxy-6-(1′,2′,3′-trihydroxypropyl)pteridine, prepared by the method of Viscontini, were phosphorylated with a mixture of phosphorus pentoxide and phosphoric acid. The products were separated by chromatography on a DEAE-cellulose column. The main phosphorylated compounds were 3′-phosphates; small amounts of 2′,3′-diphosphates were also produced. On treatment with DCC, the 3′-phosphate gave 2′,3′-cyclic phosphate.

In 1961, Goto and Forrest isolated a new phosphorylated pteridine from *E. coli*; the structure of 2-amino-4-hydroxy-6-(1′,2′,3′-trihydroxypropyl)pteridine-3′-phosphate was proposed for this compound.[1] The pteridine was synthesized in a very low yield by the condensation of 2,4,5-triamino-6-hydroxypyrimidine with D-ribose-5-phosphate[1,2] and by the reaction of 2-amino-4-hydroxy-6-(D-*erythro*-1′,2′,3′-trihydroxypropyl)pteridine (neopterin) with 2-cyanoethyl phosphate, followed by mild alkaline hydrolysis.[3] The biological function of this compound (neopterin-3′-phosphate) was proved to be that of a key compound in the biosynthesis of folic acid and the biopterin group of co-factors.[4-6] The occurrence of L-*threo*-neopterin (monapterin), and possibly its 2′,3′-cyclic phosphate, in nature has been reported.[7-9] Recently, the enzymic transformation of D-*erythro*-neopterin to D-*threo*-neopterin has been demonstrated.[10]

For the study of the biosynthesis of pteridines, we have synthesized the 3′-phosphates of D-*erythro*-, L-*erythro*-, D-*threo*-, and L-*threo*-neopterins by simplified methods; we used the phosphorylation procedure usually applied to nucleosides.[11] The neopterins were prepared by the method of Viscontini *et al.*[12] and were phosphorylated with a mixture of phosphorus pentoxide and phosphoric acid. The products were separated by chromatography on a DEAE-cellulose column. The main phosphorylated compounds were 3′-phosphates (I); small amounts of 2′,3′-diphosphates were also produced. Furthermore, the 2′,3′-cyclic phosphate of D-*erythro*-neopterin was synthesized by the action of DCC on D-*erythro*-neopterin-3′-phosphate.[13] The cyclic phosphate of D- or L-*erythro*-neopterin was recently isolated from *Photobacterium phosphoreum* (Suzuki and Goto, unpublished).

The biochemical function of the pteridines thus synthesized will be discussed elsewhere.



I

Expeirmental

2-Amino-4-hydroxy-6-(1′,2′,3′-trihydroxypropyl)pteridine.[14] D-*erythro*-, L-*erythro*-, D-*threo*-, and L-*threo*-neopterins were prepared as has been described by Viscontini *et al.*[12] D-Arabinose phenylhydrazone (8.9 g, 34.8 mmol) and 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride (6.8 g, 31.8 mmol) in 50% methanol (1600 ml, a few drops of 2-thio-

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14) Abbreviated as neopterin in this paper; neopterin is actually a D-*erythro* form of the compound.

ethanol were added) were refluxed for 3.5 hr under nitrogen. The reaction mixture was then concentrated to dryness *in vacuo*, the residue was dissolved in water (1600 m*l*), and the solution was neutralized with a 5% sodium bicarbonate solution. After aeration for 18 hr, the solution was acidified with acetic acid to pH 3—4 and acetone (30 m*l*) was added. The precipitate was collected by centrifugation and washed, in succession, with ice water, ethanol, acetone, and ether. The crude product was then dissolved in ammonia (pH 8.0) and purified by a conventional chromatographic method using a Dowex 1X8 column ($HCOO^-$, 7.5×24.0 cm) and a 0.03M ammonium formate buffer; the pH of the developer was changed continuously from 8 to 7. The reaction mixture was then separated into three fluorescent bands. The eluate of the second band was concentrated to 1 *l*, and the solution was kept in a refrigerator to give 3.0 g (37%) of D-*erythro*-neopterin. Recrystallization from water gave 1.1 g of needles.

Similarly, L-*erythro*-, D-*threo*-, and L-*threo*-neopterins were prepared by the condensation of 2,4,5-triamino-6-hydroxypyrimidine with L-arabinose-, D-xylose-, and L-xylose-phenylhydrazones respectively. The analyses, yields, and optical rotations of the products are summarized in Table 1.

Phosphorylation. D-*erythro*-Neopterin (1 mmol), 85% phosphoric acid (6 g) and phosphorus pentoxide (4 g) were mixed at 0°C, after which the mixture was stirred at 40—45°C for 1 hr. Water (60 m*l*) was added and heated for 30 min at 95—100°C. The compounds were adsorbed on charcoal (5 g); the charcoal was then washed well with water and eluted with a solution containing 50% ethanol and 4% ammonia (1 : 1). The eluted solution was concentrated to a small bulk at below 40°C; then it was put on the top of a DEAE-cellulose column (3.0×15.0 cm), which was then washed successively with 0.001N hydrochloric acid, 0.003N hydrochloric acid-0.01M lithium chloride, and 0.003N hydrochloric acid-0.1 M lithium chloride. Neopterin was eluted

Table 1. Synthesis of neopterin-analysis, yield and $[\alpha]_D$

Compound	Analysis			Yield (%)	$[\alpha]_D$ [a] (c=0.3 in 0.1N HCl)
Found:	C%	H%	N%		
D-*erythro*-Neopterin	42.60	4.25	27.66	37	+47.8±2.5° (27.5°C)
L-*erythro*-Neopterin	42.99	4.37	27.66	42	−33±3° (24.5°C)
D-*threo*-Neopterin	43.12	4.00	27.07	33	−112±2 (24.5°C)
L-*threo*-Neopterin	42.32	4.33	27.91	67	+85±2° (24.5°C)
Calcd for $C_9H_{11}N_5O_4$	42.69	4.38	27.67		

a) For Ref. see lit. 12, 15, 16, 17.

Table 2. Phosphorylation of neopterin—yield (in TOD[a])

Neopterin		Phosphorylated compound		
		neopterin 0.001 N HCl	3′-phosphate 0.003N HCl—0.01 M LiCl	2′,3′-phosphate 0.003 N HCl—0.1 M LiCl
D-*erythro*	7480	2000	1880	157
L-*erythro*	7480	2300	2310	666
D-*threo*	7480	3200	2370	500
L-*threo*	7480	1680	2400	540

a) TOD: Optical density (365 nm) of the product in 1 m*l* of 0.1—0.2 N NaOH.

Table 3. Analysis of neopterin-phosphates

Compound	Analysis			
	C%	H%	N%	P%
Found:				
D-*erythro*-Neopterin-3′-phosphate	30.64	4.09	22.19	8.47
L-*erythro*-Neopterin-3′-phosphate	30.03	3.96	21.65	7.90
D-*threo*-Neopterin-3′-phosphate	31.11	4.06	22.71	7.55
L-*threo*-Neopterin-3′-phosphate	—	—	—	—
Calcd for $C_9H_{11}O_7N_5P^- \cdot NH_4^+ \cdot H_2O$	29.35	4.62	22.83	8.42
Found:				
D-*erythro*-Neopterin-2′,3′-diphosphate	20.35	4.51	19.83	11.7
Calcd for $C_9H_{11}O_{10}N_5P_2^{2-} \cdot 2NH_4^+ \cdot 4H_2O$	20.81	5.20	18.88	11.9

15) K. J. M. Andreus, W. E. Barber, and B. P. Tong, *J. Chem. Soc., C*, **1969**, 928.
16) H. Rembold and L. Buschmann, *Chem. Ber.*, **96**, 1406 (1963).
17) M. Viscontini and R. Provenzale, *Helv. Chim. Acta*, **51**, 1500 (1968).

by the first solvent, the 3′-phosphate, by the second, and the 2′,3′-diphosphate, by the third solvent. Each eluted fraction was treated with charcoal as has been described above, and the crude material thus obtained was recrystallized from 50% ethanol. The product was obtained as ammonium salt. Likewise, the phosphorylation was carried out for L-*erythro*-, D-*threo*-, and L-*threo*-neopterin. The yields are given by total optical density (TOD), and the analytical values of the products are summarized in Tables 2 and 3.

Neopterin-3′-phosphate. On treatment with potassium metaperiodate in a 0.1 M potassium carbonate solution for 24 hr, this compound gave 2-amino-4-hydroxypteridine-6-carboxylic acid quantitatively. The phosphate (*ca.* 0.3 mg), magnesium acetate (0.005 mM), and alkaline phosphatase (10 units, from calf mucosa) in a 0.1 M tris-buffer (pH 9.5) were incubated at 37°C for 30 min; it was then hydrolyzed to neopterin theoretically. The phosphate was identical with the neopterin-3′-phosphate prepared by the condensation of 2,4,5-triamino-6-hydroxypyrimidine and D-ribose-5-phosphate; the comparison was carried out by chromatographic and electrophoretic methods.

Neopterin-2′,3′-diphosphate. On treatment with potassium periodate in a 0.1 M potassium carbonate solution for 24 hr at room temperature, neopterin-2′,3′-diphosphate remained unchanged. On treatment with alkaline phosphates, this compound gave neopterin quantitatively. This evidence indicates that the conversion of the 2′-phosphate to the 3′-phosphate occurred during the incubation process.

D-erythro-*Neopterin-2′,3′-cyclic phosphate.* Free D-*erythro*-neopterin-3′-phosphate was obtained by passing the ammonium salt (74 mg, 0.2 mM) through a Dowex 50 (H^+) column (developer: water). The effluent was evaporated to dryness *in vacuo* at below 30°C. The residue was dissolved in a mixture of triethylamine (0.03 m*l*, 0.2 mM) and water (2 m*l*). Methanol (15 m*l*) and DCC (1.03 g, 5 mM) were added, and the whole was refluxed for 30 min and evaporated to dryness. The residue was dissolved in a mixture of water (100 m*l*) and ether (50 m*l*) by shaking, and the insoluble portion was removed by filtration. The aqueous layer was evaporated to dryness *in vacuo*. The crude material thus obtained was purified further by chromatography using cellulose columns (5.0×25.0 cm) and the following developers: A, 2-propanol, 1% ammonia (2 : 1), and B, water. Recrystallization from a little water gave the faint yellow cyclic phosphate (yield, 44 mg).

Found: C, 33.30; H, 3.38; N, 20.02; P, 8.92%. Calcd for $C_9H_{10}O_6N_5P \cdot H_2O$: C, 32.43; H, 3.60; N, 21.02; P, 9.31%.

The sample was dried at 70—80°C and 0.01 mmHg for 12 hr over P_2O_5 for analysis. On treatment with 0.1 N hydrochloric acid at 95—100°C for 15 min, the cyclic phosphate gave only D-*erythro*-neopterin-3′-phosphate; this was identified by determining the optical rotation of the hydrolysis product (alkaline phosphatase). On treatment with a large excess of potassium metaperiodate in a 1 M sodium carbonate solution at room temperature for 12 hr, it remained unchanged.

The R_f-values of the pteridines are given in Table 4.

TABLE 4. PAPER CHROMATOGRAPHY AND ELECTROPHORESIS OF PTERIDINES

Compounds	R_f in Solvents[a)]					
	A	B	C	D	E	F
2-Amino-4-hydroxypteridine (pterin)	0.67	0.54	0.37	0.29	0.45	0 mm
Pterin-6-COOH	0.44	0.21	0.05	0.11	0.44	−16
D-*erythro*-Neopterin	0.69	0.41	0.21	0.10	0.61	0
L-*erythro*-Neopterin	0.69	0.41	0.21	0.10	0.61	—
D-*threo*-Neopterin	0.65	0.38	0.18	0.10	0.59	—
L-*threo*-Neopterin	0.65	0.38	0.18	0.10	0.59	—
D-*erythro*-Neopterin-3′-phosphate	0.42	0.07	0.03	0.02	0.77	−15
L-*erythro*-Neopterin-3′-phosphate	0.42	0.07	0.03	0.02	0.77	—
D-*threo*-Neopterin-3′-phosphate	0.42	0.07	0.01	0.02	0.77	—
L-*threo*-Neopterin-3′-phosphate	0.42	0.07	0.01	0.02	0.77	—
D-*erythro*-Neopterin-2′,3′-diphosphate	0.29	~0	0.01	~0	0.89	−28
D-*erythro*-Neopterin-2′,3′-cyclic phosphate	0.67	0.35	0.07	0.02	0.74	−17

a) Solvents: A, 2-propanol, 2% ammonium acetate (1 : 1); B, 2-propanol, 1% ammonia (2 : 1); C, 2-propanol, 5% boric acid (4 : 1); D, 1-butanol, acetic acid, water (4 : 1 : 1); E, 3% ammonium chloride F, distance to anode in electrophoresis; buffer: 0.05 M acetic acid-sodium acetate (pH 4.25); 30 min and 500 volt/25 cm.