Studies on Biologically Active Pteridines. I. The Synthesis of 6-(1*R*)[and (1*S*)]-(1-Hydroxyethyl)- and 6-(1*S*)[and (1*R*)]-(1,2-Dihydroxyethyl)-2-amino-4-hydroxypteridines

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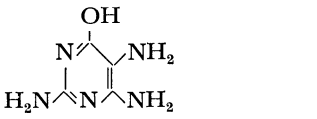
2-Amino-4-hydroxy-6-[(1*S*)-1, 2-dihydroxyethyl]pteridine has been synthesized by the condensation of 2, 4, 5-triamino-6-hydroxypyrimidine and D-threose phenylhydrazone, followed by oxidation of the intermediate carried out by adding the condensate to a mixture of $K_3[Fe(CN)_6]$, KI, and H_2O_2 in an acidic solution. The 6-(1*R*)-1, 2-dihydroxyethyl isomer and the 6-(1*R*)[and (1*S*)]-1-hydroxyethyl analogues have also been synthesized in a similar way. By applying the present method, biopterin has been obtained in 28% yield.

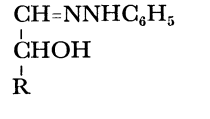
The 5,6,7,8-tetrahydro derivatives of various 2-amino-4-hydroxypteridines with an alkyl or polyhydroxyalkyl substituent at the 6-position act as a cofactor for tyrosine hydroxylase.[1,2] However, the cofactor activities of the pteridines vary depending on the structure of the side chain; tetrahydrobiopterin having an L-*erythro*-1,2-dihydroxypropyl side chain has been found to be the most active cofactor.[1,3,4] Indeed, tetrahydrobiopterin is the natural cofactor of phenylalanine hydroxylase[5] and is also thought to be the natural cofactor of tyrosine hydroxylase.[6] Recently the four stereochemical isomers of biopterin, *i.e.* 6-L-*erythro*-, 6-D-*erythro*-, 6-L-*threo*-, and 6-D-*threo*-1,2-dihydroxypropyl-2-amino-4-hydroxypteridines have been synthesized[7] and the cofactor activities of their 5,6,7,8-tetrahydro derivatives for tyrosine hydroxylase examined.[4] The L-*erythro* and D-*threo* isomers, both of which have the same configuration at the 1-position(C-1) of the side chain, exhibited similar cofactor characteristics. The D-*erythro* and L-*threo* isomers, both of which have the same, though reversed C-1 configuration compared to the others, behaved in a similar fashion as cofactor. However the latter pair were less active than the former pair. These results suggest that the cofactor activities are controlled to a fair extent by the configuration at C-1 of the side chain.[4] Consequently an examination of the cofactor characteristics of several tetrahydrobiopterin analogues having a side chain at the 6-position with an asymmetric carbon only at the 1-position(C-1) is required. This paper describes the synthesis of 6-[(1*S*)-1,2-dihydroxyethyl]- and 6-[(1*R*)-1,2-dihydroxyethyl]-2-amino-4-hydroxypteridines(**4a** and **4b**) and the 6-(1*R*)[and (1*S*)]-1-hydroxyethyl analogues(**4c** and **4d**).

Previously biopterin(**4e**) was prepared in 8% yield by the condensation of 2,4,5-triamino-6-hydroxypyrimidine(**1**) with 5-deoxy-L-arabinose phenylhydrazone (**2e**) and the subsequent oxidation of the intermediate tetrahydropteridine(**3e**).[7] This method has been one of the best methods for the synthesis of biopterin and its analogues. The first attempt to synthesize the required (1,2-dihydroxyethyl)pteridine(**4a**), in analogy to the above method, was by the condensation of **1** with D-threose phenylhydrazone(**2a**) and oxidation of the intermediate(**3a**). The main product was, however, the 6-unsubstituted 2-amino-4-hydroxypteridine(**4f**), formed by the loss of the 6-side chain during the oxidation of the tetrahydropteridine intermediate(**3a**), most probably, *via* a quinonoid dihydropteridine.[7-9] The same pteridine(**4f**) was also formed as the main product from **1** and 4-deoxy-D-erythrose phenylhydrazone (**2c**). These results suggested that the 1-hydroxyethyl or 1,2-dihydroxyethyl side chain of **3** is more susceptible to cleavage than the 1,2-dihydroxypropyl or 1,2,3-trihydroxypropyl group and hence the different oxidation conditions are required. By examining the oxidation conditions, it was found that cleavage could be reduced to a great exent by adding the solution of **3a** quickly into an aqueous acidic solution (pH 2—3) of $K_3[Fe(CN)_6]$, KI, and H_2O_2; a reverse addition, that is the addition of the oxidizing agents to the solution of **3a**, gave the 6-unsubstituted pteridine(**4f**) as the main product together with a small amount of **4a**.

Heating D-threose phenylhydrazone(**2a**) with **1** gave an orange red solution of, probably, the intermediate tetrahydropteridine(**3a**). Additional heating resulted in the development of a dark red solution, from which



1



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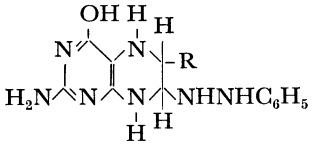

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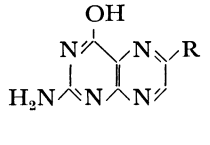
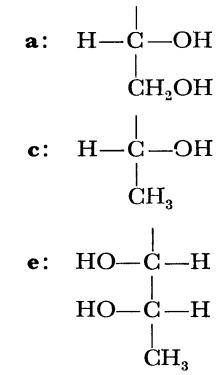
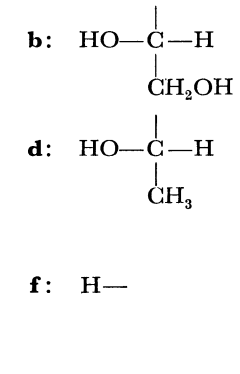


4

R=



a



b

c

d

e

f: H—

TABLE 1. THE pK_a VALUES AND UV SPECTRA OF 6-(1-HYDROXYETHYL)- AND 6-(1,2-DIHYDROXYETHYL)-2-AMINO-4-HYDROXYPTERIDINES

Compound	pK_a	pH of aqueous buffer and ionic species[a]	λ_{max} (log ε)[b]
4a	2.45±0.02	0.0 (+)	247 (3.92), 321 (3.78)
	8.05±0.02	5.5 (○)	235 (3.95), 274 (4.02), 345 (3.66)
		10.5 (−)	254 (4.24), 363 (3.73)
4b	2.41±0.02	0.0 (+)	247 (3.91), 321 (3.77)
	8.00±0.02	5.5 (○)	235 (3.92), 274 (3.99), 345 (3.65)
		10.5 (−)	254 (4.21), 363 (3.70)
4c	2.57±0.02	0.0 (+)	247 (3.89), 321 (3.75)
	8.14±0.02	5.5 (○)	235 (3.91), 273 (3.98), 345 (3.62)
		10.5 (−)	253 (4.19), 363 (3.69)
4d	2.52±0.02	0.0 (+)	246 (3.89), 320 (3.76)
	8.10±0.02	5.5 (○)	235 (3.93), 273 (3.99), 345 (3.64)
		10.5 (−)	253 (4.19), 263 (3.70)

a) Ionic species are shown by + (monocation), ○ (neutral molecule), and − (monoanion).
b) Wavelength in nm measured in aqueous buffer of given pH.

the pteridine(**4a**) could be obtained only in a poor yield. Oxidation of the intermediate(**3a**) to **4a** was most effective by adding the solution of **3a** quickly into an acidic solution of the above oxidizing agents, followed by stirring under oxygen; paper chromatograms showed that **4a** was formed as the main product together with a trace of **4f**. Column chromatographic isolation on Florisil gave **4a** as ivory needles (27%), which were shown to be free from the possible 7-substituted isomer by oxidation to the known 2-amino-4-hydroxypteridine-6-carboxylic acid by potassium permanganate.[10] The structure of the product was confirmed to be **4a** from the pK_a values and UV spectra (Table 1), which were very similar to those of biopterin (**4e**).[7]

The other three pteridines(**4b**, **4c**, and **4d**) were similarly prepared from the pyrimidine(**1**) and a corresponding tetrose phenylhydrazone(**2b**, **2c**, or **2d**). The present method was applied to the synthesis of biopterin(**4e**) which was obtained in the pure crystalline state in 28% yield.

Experimental

The elemental analyses were conducted at the Analytical Section, Meijo University, Nagoya, The pK_a values were determined spectroscopically and the UV spectra measured on a Shimadzu UV-300 spectrophotometer.

*2-Amino-4-hydroxy-6-[(1S)-1,2-dihydroxyethyl]pteridine (**4a**).* To a solution of D-*threo*-5,5-bis(ethylsulfonyl)-4-pentene-1,2,3-triol[11] (5.0 g) in water (50 ml), concentrated aqueous ammonia (0.5 ml) was added. After stirring at pH 9—10 and at room temperature for 10 h, the resulting suspension was adjusted to pH 3—4 with formic acid and filtered. Phenylhydrazine (1.8 g) was added to the filtrate and the solution stirred at pH 2—3 and 25 °C for 1 h. The solution of the formed phenylhydrazone (**2a**) was added to a solution of 2,4,5-triamino-6-hydroxypyrimidine (**1**) dihydrochloride[12] (2.5 g) dissolved in a mixture of water (100 ml) and methanol (150 ml). The solution was stirred under nitrogen at 25 °C for 1 h and then under reflux for 20 min. The orange red solution, after being chilled in an ice bath, was poured into an ice chilled aqueous solution (150 ml) containing potassium hexacyanoferrate(III) (15 g), potassium iodide (2.5 g), 35% hydrogen peroxide (10 ml), and formic acid (5 ml). The mixture was stirred vigorously under bubbling oxygen at 5—10 °C for 1 h and at 25—30 °C for 3 h. The mixture was evaporated to dryness under reduced pressure and the residue extracted with 2% aqueous ammonia (500 ml). The extract was concentrated to about 200 ml under reduced pressure, adjusted to pH 2—3 with hydrochloric acid, and placed on a Florisil column (4.5×35 cm). The column was first eluted with 0.25 M formic acid (4 l) until the inorganic salts, blue colored materials, and small amount of blue fluorescent compounds were removed from the column. The column was then eluted with water (4 l). Evaporation of the eluate under reduced pressure gave a solid which was extracted with 2% ammonia (150 ml). The extract was evaporated to dryness under reduced pressure and the residue extracted with 2% ammonia (150 ml). Concentration of the extract to about 70 ml under reduced pressure and acidification with formic acid (pH 3—4) gave a solid, which crystallized from water to give ivory needles (710 mg) of **4a**, mp> 300 °C (Found: C, 41.98; H, 3.98; N, 30.85%. Calcd for $C_8H_9N_5O_3 \cdot 0.3H_2O$: C, 41.98; H, 4.24; N, 30.61%).

*2-Amino-4-hydroxy-6-[(1R)-1,2-dihydroxyethyl]pteridine (**4b**).* L-Arabinose diethyl dithioacetal[13] (5.0 g) was degraded to L-erythrose according to the method of Hough and Taylor[11] by oxidation to L-*erythro*-5,5-bis(ethylsulfonyl)-4-pentene-1,2,3-triol with aqueous peroxypropionic acid and by subsequent treatment with dilute ammonia. After treatment with phenylhydrazine, condensation of the resulting L-erythrose phenylhydrazone (**2b**) with **1** in a similar manner to that for **4a** gave the (1*R*)-1,2-dihydroxyethyl compound (**4b**) as ivory needles in 22% yield, mp>300 °C (Found: C, 41.98; H, 3.95; N, 30.77%. Calcd for $C_8H_9N_5O_3 \cdot 0.3H_2O$: C, 41.98; H, 4.24; N, 30.61%).

*2-Amino-4-hydroxy-6-[(1R)-1-hydroxyethyl]pteridine (**4c**).* 5-Deoxy-D-arabinose diethyl dithioacetal (5.5 g), prepared according to the method of Green and Rembold[13] by lithium aluminium hydride reduction of 5-tosyl-D-arabinose diethyl dithioacetal, was degraded to 4-deoxy-D-erythrose as above. The erythrose was treated with equimolar phenylhydrazine (2.4 g) and the resulting phenylhydrazone (**2c**; unisolated) was condensed with **1** (dihydrochloride, 4.5 g) and subsequently oxidized in the same way as that used for **4a** to give ivory needles (650 mg, 15% yield) of **4c**, mp>300 °C (from water)(Found: C, 43.33; H, 4.61; N, 31.82%. Calcd for

$C_8H_9N_5O_2 \cdot 0.8H_2O$: C, 43.35; H 4.87; N, 31.61%).

*2-Amino-4-hydroxy-6-[(1S)-1-hydroxyethyl]pteridine (**4d**).* By replacing 5-deoxy-D-arabinose diethyl dithioacetal by 5-deoxy-L-arabinose diethyl dithioacetal[13] in the foregoing procedure, the (1*S*)-1-hydroxyethyl compound (**4d**) was obtained as ivory needles in 9% yield, mp >300 °C (from water) (Found: C, 42.54; H, 4.48; N, 30.86%. Calcd for $C_8H_9N_5O_2 \cdot H_2O$: C, 42.66; H, 4.93; N, 31.10%).

*Biopterin (**4e**).* The condensation of 2,4,5-triamino-6-hydroxypyrimidine(**1**) dihydrochloride (5.0 g) and 5-deoxy-L-arabinose phenylhydrazone (**2e**) (5.5 g), followed by oxidation and chromatographic isolation in a manner similar to that used for **4a**, gave biopterin (1.56 g, 28% yield) as colorless needles.

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References

1) A. R. Brenneman and S. Kaufman, *Biochem. Biophys. Res. Commun.*, **17**, 177 (1964).
2) T. Nagatsu, K. Mizutani, I. Nagatsu, S. Matsuura, and T. Sugimoto, *Biochem. Pharmacol.*, **19**, 1945 (1972).
3) Y. Numata, K. Ikuta, T. Kato, T. Nagatsu, T. Sugimoto, and S. Matsuura, *Biochem. Pharmacol.*, **24**, 1998 (1975).
4) Y. Numata, T. Kato, T. Nagatsu, T. Sugimoto, and S. Matsuura, *Biochem. Biophys. Acta*, **480**, 104 (1977).
5) S. Kaufman, *Proc. Natl. Acad. Sci. U.S.A.*, **50**, 1085 (1963).
6) S. Kaufman, "Frontiers in Catecholamine Research," ed by E. Usdin and S. H. Snyder, Pergamon Press, Oxford (1973), pp. 53—60.
7) T. Sugimoto and S. Matsuura, *Bull. Chem. Soc. Jpn.*, **48**, 3767 (1975).
8) M. Viscontini and E. Möhlmann, *Helv. Chim. Acta*, **42**, 836 (1959).
9) S. Matsuura, T. Sugimoto, C. Kitayama, and M. Tsusue, *J. Biochem. (Tokyo)*, **83**, 19 (1978).
10) R. Tschesche, B. Hess, I. Ziegler, and H. Machleit, *Justus Liebigs Ann. Chem.*, **658**, 193 (1962).
11) L. Hough and T. J. Taylor, *J. Chem. Soc.*, **1955**, 1212.
12) T. Sugimoto and S. Matsuura, *Research Bulletin (Dept. of General Education, Nagoya Univ.)*, **12**, 24 (1968).
13) B. Green and H. Rembold, *Chem. Ber.*, **99**, 2162 (1966).