ENZYMATIC OXIDATION OF A DERIVATIVE OF 6-HYDROXYPTERIDINE *

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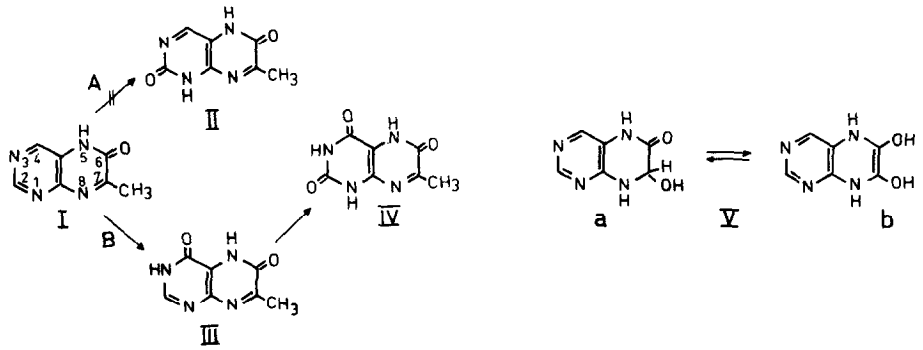
Among all hydroxypteridines only the 6-hydroxy- and the 2,6-dihydroxy derivatives are refractory towards milk xanthine oxidase (XO) (Bergmann and Kwietny, 1958, 1959). In both these pteridines, the $-\overset{7}{C} = \overset{8}{N}-$ bond hydrates reversibly to -CHOH-NH- (as in V a) (Albert, 1955; Brown and Mason, 1956 ; Albert, Lister and Pedersen, 1956). Bergmann and Kwietny (1959) assumed therefore that the strongly polar 6-hydroxyl group interferes with the dehydrogenation of the neighboring 7-CHOH-group to carbonyl. Inoue and Perrin (1962) suggested that hydration destroys the planar structure of the pteridine skeleton and thus hampers adsorption onto a flat portion of the active center (e.g. the flavin moieties) of the enzyme molecule.



Hydration at position 7,8 is precluded by methylation at C-7 (Inoue and Perrin, 1962). We have therefore tested 6-hydroxy-7-methylpteridine (I) (Albert and Reich, 1961) as substrate of XO. Already during the first 10 minutes a slight shift of λ_{max} towards longer wavelengths took place, and the solution , which in the beginning was completely transparent, acquired a brilliant blue-green

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fluorescence when viewed under an ultraviolet lamp. Later on the fluorescence became so intense as to be visible in daylight. After about 20 hrs. the absorption maximum had shifted from 349 to 380 mμ. Comparison of the properties of the end-product, after purification by paper chromatography, with those of 2,4,6-trihydroxypteridine (Table I) indicates the formation of the 7-methyl derivative (IV).

TABLE I. PROPERTIES OF PTERIDINES

Compound	λ_{max} (mμ) at pH 8.0	R_F	Fluorescence
6-Hydroxypteridine	358	0.49	Black violet
6-Hydroxy-7-methylpteridine (I)	348/9	0.65	Brown
2,6-Dihydroxypteridine	298	0.11	Green
4,6-Dihydroxypteridine	359	0.15	Blue
4,6-Dihydroxy-7-methylpteridine (III)	343	0.57	Blue
2,4,6-Trihydroxypteridine	381	0.07	Blue green
2,4,6-Trihydroxy-7-methylpteridine (IV)	380	0.08	Brilliant blue

R_F values were determined by descending paper chromatography using the following solvent : 95% ethanol:25% ammonia:water = 80:10:10, by vol. Spots were located by their fluorescence under an ultraviolet lamp, emitting light of about 255 mμ. Oxidation took place under the following conditions : Milk xanthine oxidase (Nutritional Biochemical Corporation, Cleveland, Ohio) 1:200 (this enzyme, at a dilution of 1:1600, produces 9.7 μmoles/min/l of uric acid, when xanthine 65 uM serves as a substrate) ; 6-hydroxy-7-methylpteridine, 13 μg/ml ; 0.01M TRIS buffer of pH 8.0 ; temperature 30° ; catalase (Worthington Biochemical Corporation , Freehold, New Jersey) 1:1500 .

In order to determine whether the reaction follows pathway A or B, samples for paper chromatography were withdrawn every 2 min. After about 20 min a spot with R_F 0.57, blue fluorescence and λ_{max} 343 mμ appeared. This material might represent 7-methyl-4,6-dihydroxypteridine (III) (see Table I); however final identification must be postponed, until synthetic III will become available. Nevertheless, in view of the fact that C-methylation does not alter much the

absorption spectra of pteridines (Albert and Howell, 1962), it may be assumed that the alternative intermediate, viz. 7-methyl-2,6-dihydroxypteridine (II), would exhibit a maximum somewhat below 298 mμ. Accordingly, formation of II should have caused an initial hypsochromic shift during enzymatic attack on I.

Although the rate of oxidation of I is less than 1% of that of xanthine, the present results demonstrate beyond doubt that the 7-methyl group breaks the resistance of 6-hydroxypteridine towards XO. On the other hand, pteridine and its 2-hydroxy derivative, which undergo hydration at the 3,4-double bond (Perrin, 1962; Brown and Mason, 1956; Albert and Howell, 1962), are slowly oxidised by XO (Bergmann and Kwietny, 1959). It therefore appears possible that non-planarity of a substrate, resulting from hydration, may not be the sole factor responsible for obstruction of enzymatic attack and that hydration at position 7,8 may exert a specific inhibitory influence. Indeed, hydration of 6-hydroxypteridine can give rise to formation of an enediol (Vb). This structure may form an ES-complex which cannot undergo dehydrogenation by XO. The problem of a specific role of the hydrated 7,8-azomethine group in 6-hydroxylated pteridines may be attacked by studying other C-methyl derivatives of 2- and 6-hydroxy- and 2,6-dihydroxypteridine.

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