IDENTIFICATION OF A NEW PHOSPHORYLATED PTERIDINE FROM E. COLI[1]

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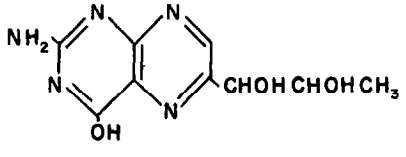
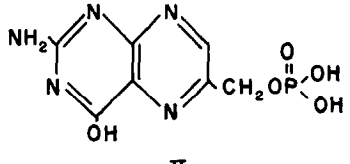
Received October 12, 1961

It is commonly supposed that the pteridine ring is biosynthesized from a purine precursor although the evidence for this is meager except in the case of riboflavin. Recently Vieira and Shaw (1961) using the bacterium Corynebacterium sp have demonstrated the incorporation of adenine-2-C^{14} into a folic acid compound (teropterin) which on degradation yielded labelled 2-amino-4-hydroxypteridine. Obviously, however, adenine is not the immediate precursor of the pteridine ring, and various other more likely purines have been proposed. These include guanosine, which could be converted into a compound of the biopterin type (cf. I) by loss of C-8 and subsequent ring closure using two of the carbon atoms of the ribosyl group to complete the pyrazine ring (Forrest, 1960). Recently Weygand et al. (1961) have suggested guanosine-5'-phosphate



I



II

[1] This work was supported by a PHS research grant, RG-6492, from the National Institutes of Health, Public Health Service and by the Robert A. Welch Foundation, Houston, Texas.

as a precursor of leucopterin (2-amino-4, 6, 7-trihydroxypteridine) in the butterfly, Pieris brassicae L., the first pteridine intermediate postulated being a (2-amino-4-hydroxy-6-pteridinyl)glycerol phosphate* derivative. A compound of this type could conceivably be a folic acid intermediate also, either as a dihydro or tetrahydro derivative or after conversion into (2-amino-4-hydroxy-6-pteridinyl)methyl phosphate (II) by degradation and resynthesis. This latter compound, probably again as a reduced derivative, has been postulated as an intermediate in the scheme proposed by Shiota and Disraely (1960) for the biosynthesis of folic acid; a dihydro derivative of the unphosphorylated compound has been suggested by Brown (1961); and a dihydro derivative with a pyrophosphate group on the side chain has been proposed by Jaenicke and Chan (1960).

We wish to report the isolation from Eschericha coli in very small amount of a compound whose properties are consistent with its formulation as (2-amino-4-hydroxy-6-pteridinyl)glycerol phosphate* (III). E. coli cells (wet weight, 1 Kg.) were extracted with boiling water (5 l.) and centrifuged. The supernatant was acidified to pH 3 and treated with charcoal (16 g.). The adsorbed fluorescent compounds were eluted from this with a mixture of 4% aqueous ammonia and ethanol (3:1). The eluate was evaporated to dryness in vacuo, below 50° C., and the brown residue (2.5 g.) was purified by paper chromatography using the solvent 4% sodium citrate-isoamyl alcohol.

A broad blue-fluorescent band (Rf 0.7-0.9) was eluted with water, and the eluate was again subjected to treatment with charcoal. After elution of the fluorescent material from this in the same way as before, further purification was achieved by successive paper chromatography with the solvents n-propanol-1% aqueous ammonia (2:1) (the fluorescent band of Rf 0.11 being cut out and eluted) and

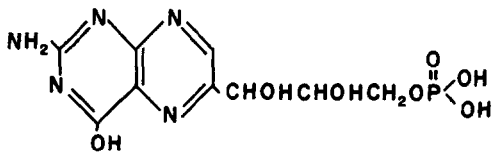
* More systematically named 3-(2-amino-4-hydroxy-6-pteridinyl)-2, 3-dihydroxypropyl dihydrogen phosphate.

n-butanol-acetic acid-water (4:1:1) (the band of Rf 0.05 being eluted). The chemical constitutions of the other fluorescent compounds, which represented the main bulk of extracted fluorescent compounds and which were separated by these procedures, have not yet been determined, but evidence is presented below of the structure of the compound running as a fluorescent band in the solvents used and characterized by the Rf values given above.

This blue-fluorescent compound (obtained in a yield of less than 1 mg.) has an absorption spectrum identical with that of a 2-amino-4-hydroxy-6-alkylpteridine and was oxidized with alkaline permanganate into 2-amino-4-hydroxy-6-carboxypteridine which was characterized by chromatographic and electrophoretic comparison with authentic material. It behaved electrophoretically as an acid and, on incubation with an alkaline phosphatase preparation (N. B. Co.), yielded 2-amino-4-hydroxy-6-trihydroxypropylpteridine, again as determined by comparison chromatographically and electrophoretically with an authentic sample. The original compound was oxidized by sodium metaperiodate to yield 2-amino-4-hydroxy-6-formylpteridine indicating that the blocked hydroxyl group was attached to the terminal carbon atom of the side chain. Finally, a compound chromatographically and electrophoretically identical with the E. coli compound was obtained in low yield by condensation of 2, 4, 5-triamino-6-hydroxypyrimidine with ribose-5-phosphate pretreated with p-toluidine (Weygand, Wacker, and Schmied-Kowarzik, 1949).

Although complete identification of this compound must await its isolation in quantity and its unambiguous synthesis, the evidence presented above strongly suggests that its structure can be written as shown in III.

The biological function of this compound is not yet known. It may, for example, be a catabolic product of pteridine metabolism.



III

However, its structural features strongly suggest that it, or a reduced derivative, is an intermediate in pteridine biosynthesis; in addition it may be a key link between the biopterin group of pteridines (biopterin, sepiapterin, drosopterin, etc.) and the folic acid group. Further work along the lines suggested above is in progress on the chemistry of this compound and its biological activity.

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