ORIGIN OF THE SIDE-CHAIN IN PTERIDINES OF THE BIOPTERIN TYPE

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It has been suggested (Forrest and Nawa, 1964) that, in the biosynthesis of biopterin and biopterin glucosides, the three-carbon side-chain, presumed to be present in the pteridine arising from guanylic acid, is cleaved and replaced by a new three-carbon unit arising from α-ketobutyric acid. This paper reports experiments in a blue-green alga which confirm this suggestion. Threonine-U-C^{14} is incorporated into biopterin glucoside in growing cultures of Anacystis nidulans. Some of the radioactivity is present in the glucose moiety, but after its removal, followed by oxidation of the resulting biopterin to 2-amino-4-hydroxypteridine-6-carboxylic acid, approximately one-third of the radioactivity in the biopterin is still associated with the pteridine carboxylic acid.

EXPERIMENTAL

Anacystis nidulans was grown for 18 hr. at 38.5° in medium C of Kratz and Myers (1956) (16 ml.) containing 0.01 mc. DL-threonine-U-C^{14}. Air, enriched with CO_2 (3-1/2%), was bubbled through the culture. Turbidometric measurement showed a factor increase in cell mass of 340. Compounds were purified by paper chromatography in three solvent systems; butanol-acetic acid-water (4:1:5), propanol-ammonia 1% (2:1) and 4% ammonia and determined fluorometrically with a Farrand Fluorometer (Model A). Radioactivity was measured with a gas flow counter.

Extraction of biopterin glucoside. At the end of the growth period the cells were harvested, suspended in 0.1 M acetic acid (10 ml.), manganese dioxide

was added, and the suspension was allowed to stand for 1 hr. It was then centrifuged and the residue was washed twice with 0.1 M acetic acid (10 ml.). The supernatant and washings were combined and charcoal was added to adsorb all fluorescence; after washing, the charcoal was extracted with propanol-conc. ammonia (2:1). The blue fluorescent eluate containing biopterin glucoside was purified by paper chromatography (3 solvents).

Biopterin glucoside was converted into biopterin by heating on a steam bath for 6 hr. in N HCl. The resulting biopterin was adsorbed on charcoal and purified by paper chromatography (3 solvents).

Biopterin was converted into 2-amino-4-hydroxypteridine-6-carboxylic acid by oxidation with alkaline potassium permanganate on a steam bath for 30 min., and the product separated from the reaction mixture by chromatography in butanol-acetic acid-water; it was then purified by paper chromatography (3 solvents). Two experiments were done, in one biopterin glucoside was oxidized directly to 2-amino-4-hydroxypteridine-6-carboxylic acid. The results of these experiments are shown in Table 1.

Table 1

Compound	cpm/μmole Experiment 1	cpm/μmole Experiment 2
Biopterin glucoside	148	49.4
Biopterin	—	31.4
Pteridine-6-carboxylic acid	50.6	14.3

DISCUSSION

The evidence presented above demonstrates that a three-carbon unit derived from threonine is incorporated into biopterin glucoside. A reasonable assumption is that the threonine gives rise to α-ketobutyric acid which then undergoes decarboxylation to give "active propionaldehyde" and this in turn is added to

reduced 2-amino-4-hydroxypteridine. This latter reaction has been shown to occur non-enzymatically (Forrest and Nawa, 1964).

Two other pieces of evidence support this concept. 1) Reduced, radioactive 2-amino-4-hydroxypteridine has been shown to be incorporated into the drosopterins (the red eye pigments from Drosophila) (Goto, Okada, and Forrest, 1964) which also contain a three-carbon side-chain. 2) Neopterin, which may be presumed to arise from (2-amino-4-hydroxy-6-pteridinyl)glycerol phosphate, has the D-erythro configuration (Rembold and Buschmann, 1963), whereas biopterin has the L-erythro configuration, suggesting that the glycerol side-chain is replaced in toto.

An alternative origin of the three-carbon side-chain (Trebst, 1964) in which a two-carbon unit is joined to 2-amino-4-hydroxy-6-formylpteridine is ruled out by the experiments reported herein.

The possibility exists that carbon 9 in folic acid-like compounds arises by a similar mechanism to the one described above, involving cleavage, and addition of a one-carbon unit to the reduced 2-amino-4-hydroxypteridine. In fact this would seem to be a satisfactory explanation of the demonstration by Jones,

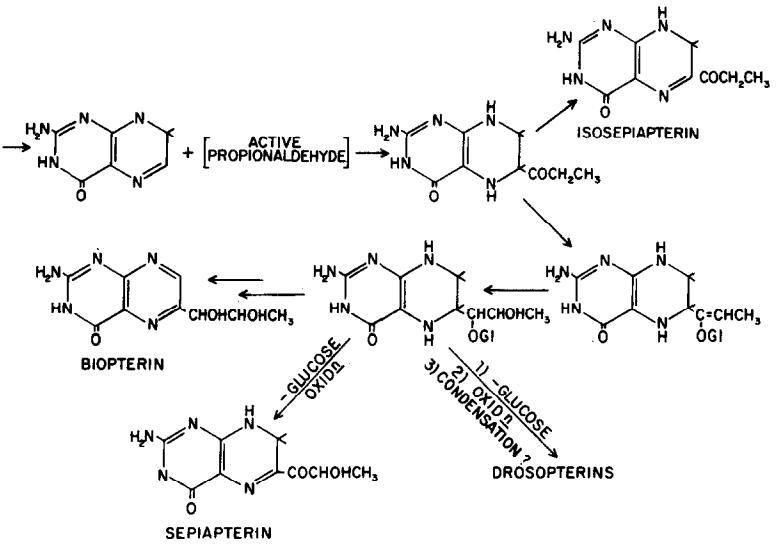

Figure 1. Hypothetical scheme for interrelationships between pteridines with three-carbon side-chains in the 6-position.

Reynolds, and Brown (1964) that all four possible optical isomers of the glycerol side-chain are more or less active in pteroic acid biosynthesis.

A plausible mechanism for the derivation of all of the naturally occurring pteridines containing a three-carbon side-chain at the 6-position is outlined in Figure 1.

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