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FOLIC ACID

Supplement*

SYNTHESIS OF PTEROYLGLUTAMIC ACID (LIVER *L. CASEI* FACTOR) AND PTEROIC ACID—PART II

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SYNTHESIS OF PTEROYLGLUTAMIC ACID (LIVER *L. CASEI* FACTOR) AND PTEROIC ACID— PART II

The method of synthesis of pteroic acid derivatives to be presented here was suggested by descriptions in the literature of quaternary ammonium salts as alkylating agents. Snyder, Smith, and Stewart[1] describe the use of benzyltrimethylammonium salts and dimethylaminomethylindole (gramine) in the alkylation of malonic and similar esters to yield the corresponding C-benzyl derivatives or substituted esters of *beta*-(3-indole) propionic acid.

These and other workers later made use of this type of reaction in the preparation of synthetic *dl*-tryptophane.[2, 3, 4]

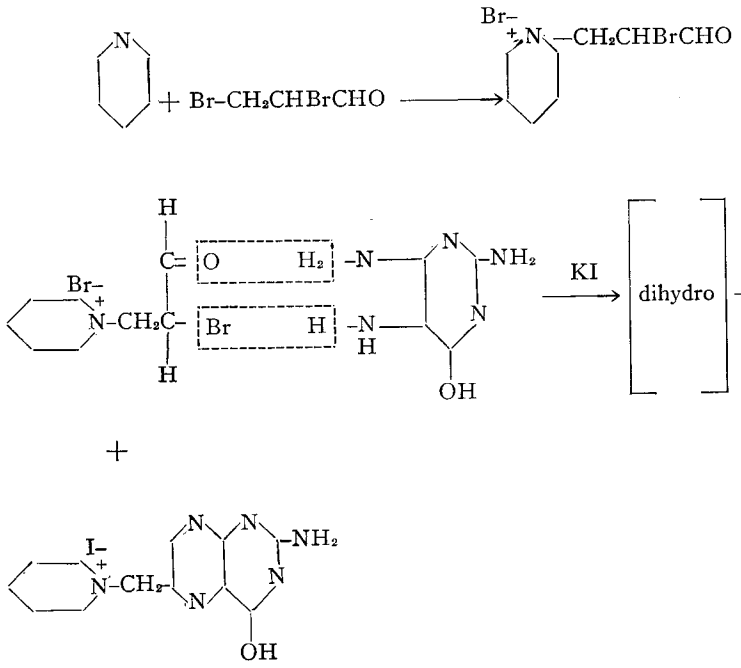
The use of simple quaternary ammonium salts in the preparation of sulfur[5] and oxygen[6-11] alkylated derivatives has also been described.

The use of a tertiary amine to alkylate another amine is reported by Howe, Zambito, Snyder, and Tishler.[4] The effectiveness of pyridine as the tertiary base component of the quaternary ammonium salt, as an alkylating agent, was shown by Snyder and Speck.[5]

Our efforts were therefore directed at the preparation of a suitable pterin for the alkylation of *p*-aminobenzoylglutamic acid to obtain the desired pteroylglutamic acid. The successful use of 2,3-dibromopropionaldehyde in the synthesis, described in the previous paper, gave an indication of an intermediate which might be suitable. It was found that the addition of pyridine to a cooled solution of 2,3-dibromopropionaldehyde in ether gradually gave a precipitate of a crystalline quaternary salt. This same reaction could be carried out in somewhat lower yield in aqueous solution. The quaternary salt was filtered, or extracted from the ether layer with water, and the ether solution was discarded. The resulting water solution was then added to a solution of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride and potassium iodide in water.

The potassium iodide serves to precipitate the less soluble iodide salt. On standing overnight at room temperature, some separation of product occurred, and, on adjusting the pH to about 3.0, more crystalline

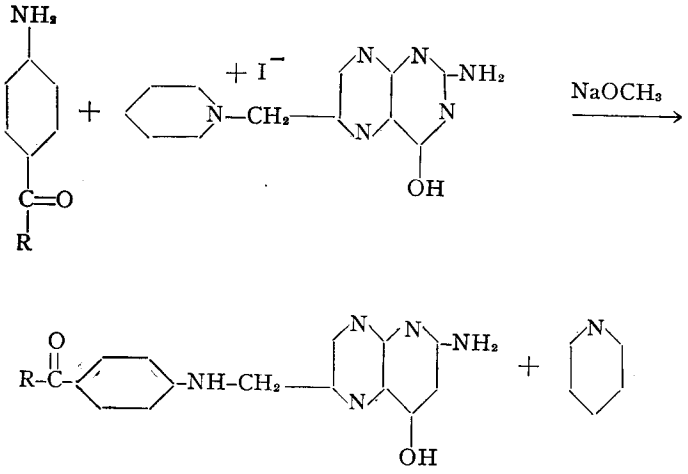
material and some brown, amorphous material separated. This crude material was purified by recrystallization from water, using activated charcoal as a decolorizing agent. The resulting purified N[(2-amino-4-hydroxy-6-pteridyl)-methyl]pyridinium iodide occurred as thin, lenticular crystals, often resembling those of pteroylglutamic acid itself. The analysis for nitrogen and iodine gave values corresponding to those expected for $C_{12}H_{11}IN_6O$.



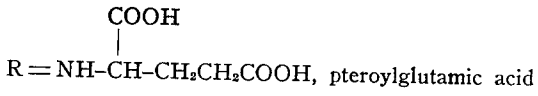
Oxidation of this compound, N[(2-amino-4-hydroxy-6-pteridyl) methyl]pyridinium iodide, with hot, alkaline permanganate solution yielded the corresponding 2-amino-4-hydroxypteridine-6-carboxylic acid, which showed the substituted methyl group to be in the 6- position.

To prepare the pteroylglutamic acid, a mixture of N[(2-amino-4-hydroxy-6-pteridyl)methyl]pyridinium iodide, *p*-amino-benzoylglutamic acid, and sodium methylate in ethylene glycol solution was heated to about 140° C. for three hours.

This reaction mixture on dilution with water and acidification to pH 3.0-3.5 gave a product containing about 15 per cent of the biologically active material.



where R = OH, pteroic acid



R = NH–CH(COOH)–CH_2CH_2COOH, pteroylglutamic acid

Purification of this material, as previously described, gave a product having the physical and biological properties described in a previous publication.[12]

By the use of *p*-aminobenzoic acid in place of *p*-amino-benzoylglutamic acid, a compound was obtained which was active for *Streptococcus faecalis* R but inactive for *Lactobacillus casei* and the chick.

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