[CONTRIBUTION FROM LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, AND CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Synthesis of Pteroylglutamic Acid. IV

BY JAMES H. BOOTHE,[1] COY W. WALLER,[1] E. L. R. STOKSTAD[1], BRIAN L. HUTCHINGS,[1] JOHN H. MOWAT,[1] ROBERT B. ANGIER,[1] JOSEPH SEMB,[1] Y. SUBBAROW,[1] DONNA B. COSULICH,[2] M. J. FAHRENBACH,[2] M. E. HULTQUIST,[2] ERWIN KUH,[2] E. H. NORTHEY,[2,3] DORIS R. SEEGER,[2] J. P. SICKELS[2] AND JAMES M. SMITH, JR.[2]

The structure of pteroylglutamic acid requires the presence of a methylene group connecting the pteridine nucleus and N-(*p*-aminobenzoyl)-glutamic acid. The methods of synthesis previously reported[4] have made use of the reaction of a three-carbon compound between 2,4,5-triamino-6-hydroxypyrimidine and N-(*p*-aminobenzoyl)-glutamic acid, or the alkylation of N-(*p*-aminobenzoyl)-glutamic acid with 2-amino-4-hydroxy-6-pteridylmethylpyridinium iodide.

This communication deals with the halogenation of 2-amino-4-hydroxy-6-methylpteridine, and the alkylation of N-(*p*-aminobenzoyl)-glutamic acid with the crude halomethylpteridine. It includes an improved method of synthesis of 2-amino-4-hydroxy-6-methylpteridine and a description of the dihydro form of this compound.

The previously described method of preparation of 2-amino-4-hydroxy-6-methylpteridine[5] was difficult to adapt to large scale preparative work. A more convenient method of preparing this pteridine was found to be by the reduction of 2-amino-4-hydroxy-6-pteridylmethylpyridinium iodide.[4b] The reduction was accomplished by dissolving the compound in 1 *N* sodium hydroxide and stirring with zinc dust. During the reduction the pteridine ring was reduced to a dihydro form which could be oxidized readily to the aromatic form with hydrogen peroxide, iodine or potassium permanganate. It was proved to be a dihydro form and not a more highly reduced form by oxidation to 2-amino-4-hydroxy-6-methylpteridine with the theoretical amount of potassium permanganate. This dihydro compound has an ultraviolet absorption spectrum quite different from the ordinary aromatic form of the pteridines (Fig. 1).

Several methods of halogenation have been used with varying degrees of effectiveness. Bromination was accomplished by heating the 2-amino-4-hydroxy-6-methylpteridine in a sealed tube with bromine at temperatures of from 100–150°. Catalytic amounts of benzoyl peroxide and concentrated hydrochloric acid were each tried but both resulted in lowered yields. The most effective temperature seemed to be 150°. Bromination was also accomplished by heating the methyl-

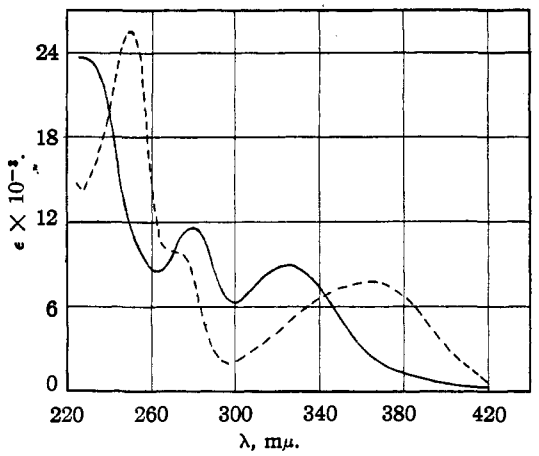
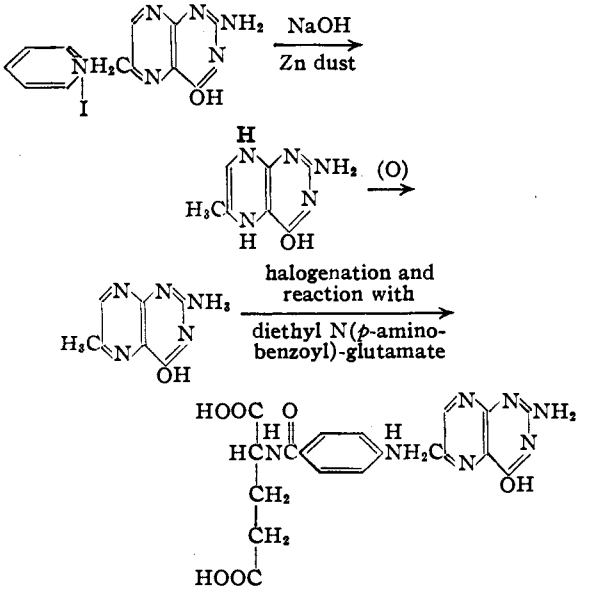


Fig. 1.—Ultraviolet absorption spectra of 2-amino-4-hydroxy-6-methyldihydropteridine hydrochloride monohydrate: ——— in 0.1 *N* sodium hydroxide; - - - - - - in 0.1 *N* hydrochloric acid.

pteridine in 48% hydrobromic acid and bromine. Chlorination was effected to a very limited extent by refluxing the methylpteridine in sulfuryl chloride and benzoyl peroxide. These crude halogenation products then reacted with diethyl N-(*p*-aminobenzoyl)-glutamate[4a] by heating in ethylene glycol at 100–110°. The products of this condensation were then assayed microbiologically



(1) Lederle Laboratories Division, American Cyanamid Company.

(2) Calco Chemical Division, American Cyanamid Company.

(3) Present address: Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

(4) (a) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948). (b) Hultquist, *et al.*, *ibid.*, **70**, 23 (1948). (c) Angier, *et al.*, *ibid.*, **70**, 25 (1948).

(5) Mowat, *et al.*, THIS JOURNAL, **70**, 14 (1948).

against *S. faecalis* R after hydrolysis of the ester groups.

Experimental

2-Amino-4-hydroxy-6-methyldihydropteridine.—Ten grams of 2-amino-4-hydroxy-6-pteridylmethylpyridinium iodide was dissolved in 500 cc. of 1 *N* sodium hydroxide. Twenty grams of zinc dust was added and the reaction mixture was stirred twenty minutes at room temperature. The excess zinc was filtered out and the solution was adjusted to *p*H 2.7 with concentrated hydrochloric acid. The light yellow precipitate was removed by filtration and dried; weight was 6 g. This product contained some zinc oxide. It was purified by dissolving in the minimum amount of 0.5 *N* sodium hydroxide, treating with Norite, and then adding an equal volume of 10 *N* sodium hydroxide solution. On cooling, the sodium salt crystallized and was removed by filtration. The sodium salt was then dissolved in the minimum amount of warm water and enough concentrated hydrochloric acid was added to make the solution 0.5 *N*. The yellow crystalline hydrochloride separated and was filtered out. For an analytical sample this purification was repeated once more. The hydrochloride crystallizes as a monohydrate, from which water is not removed by heating at 60° *in vacuo*.

Anal. Calcd. for $C_7H_{10}ON_5 \cdot HCl \cdot H_2O$: C, 36.05; H, 5.14; N, 30.00. Found: C, 36.20; H, 5.47; N, 30.20.

2-Amino-4-hydroxy-6-methylpteridine.—(a) Two hundred mg. of 2-amino-4-hydroxy-6-methyldihydropteridine was dissolved in 10 cc. of dilute sodium hydroxide. A solution of 0.2 *M* potassium permanganate was added dropwise at room temperature until a light green color persisted when the manganese dioxide was centrifuged out. This required 3.7 cc. After discharging the green color with sodium sulfite, the manganese dioxide was filtered out and the clear yellow solution was acidified with hydrochloric acid. The precipitated product was centrifuged and washed with water. It was crystallized by dissolving in 6 cc. of dilute sodium hydroxide and adding 6 cc. of 10 *N* sodium hydroxide. The crystalline sodium salt was dissolved in water and the solution was acidified. The product was collected and dried. Weight was 130 mg. The ultraviolet absorption spectrum was identical to that of authentic samples of 2-amino-4-hydroxy-6-methylpteridine.[5]

(b) One hundred grams of 2-amino-4-hydroxy-6-pteridylmethylpyridinium iodide was dissolved in 4 liters of 1 *N* sodium hydroxide and 100 g. of zinc dust was added. The mixture was stirred twenty minutes and the excess zinc was filtered out. Sixteen cc. of 30% hydrogen peroxide was added and stirred ten minutes at room temperature. The solution was then heated on the steam-bath and treated with Norite. The clarified solution was then added dropwise to a heated, stirred solution of 600 cc. of acetic acid in 2 liters of water. After standing overnight the supernatant liquid was siphoned off and the product collected on a filter, washed and dried. Weight was 32.4 g. This material was pure enough for most work but could be crystallized from 5 *N* sodium hydroxide as described above. For an analytical sample the material was crystallized twice from boiling water.

Anal. Calcd. for $C_7H_7N_5O$: C, 47.45; H, 3.96; N, 39.6. Found: C, 47.40; H, 4.32; N, 39.45.

The ultraviolet and infrared absorption spectra were identical with authentic samples of 2-amino-4-hydroxy-6-methylpteridine.[5]

Pteroylglutamic acid.—(a) Two grams of 2-amino-4-hydroxy-6-methylpteridine was sealed in a glass tube with 2.5 g. of bromine. The tube was heated at 150–155° for five hours, cooled and opened. The contents were dried in vacuum over potassium hydroxide for twenty-four hours and then washed out of the tube with a solution of 1 g. of diethyl N-(*p*-aminobenzoyl)-glutamate in 100 cc. of hot ethylene glycol. This mixture was heated one hour at 100–110°, cooled, and diluted with 300 cc. of ethanol. The insoluble product was removed by centrifuging, washed with alcohol and ether, and dried. Weight was 1.75 g. After hydrolysis of the ester groups by standing in 0.1 *N* sodium hydroxide for twelve hours, the crude material was shown to contain 14% pteroylglutamic acid by microbiological assay against *S. faecalis* R. The active material was isolated by the method previously described[6] and found to be identical with pteroylglutamic acid by comparison of the microbiological assay, the ultraviolet and infrared absorption spectra.

(b) Twelve grams of 2-amino-4-hydroxy-6-methylpteridine was suspended in 500 cc. of 48% hydrobromic acid and 12 cc. of bromine was added. The suspension was heated on a steam-bath under a reflux condenser with occasional stirring for five hours. The methylpteridine was nearly all in solution at this point. After standing overnight at room temperature, the solution was concentrated in vacuum until all the excess bromine had been removed. The solution was then treated with Norite, filtered, and concentrated in vacuum until the product began to crystallize (30–40 cc.). To this concentrated solution was added 2.5 liters of cold water which precipitated the product. It was filtered and dried; weight was 14 g. One hundred mg. of this material was heated three hours at 100° with 400 mg. of diethyl N-(*p*-aminobenzoyl)-glutamate in 8 cc. of ethylene glycol. The solution was cooled and diluted with 30 cc. of acetone. The insoluble product was centrifuged, washed and dried. Weight was 60 mg. After hydrolysis of the ester groups in 0.1 *N* sodium hydroxide at room temperature for twelve hours, the material was bioassayed with *S. faecalis* R and found to contain 14.8% pteroylglutamic acid.

(c) Five hundred mg. of 2-amino-4-hydroxy-6-methylpteridine was suspended in 10 cc. of sulfuryl chloride and a small amount of benzoyl peroxide was added. The mixture was refluxed five hours, evaporated to dryness and dry benzene was added and evaporated to dryness again. One gram of diethyl N-(*p*-aminobenzoyl)-glutamate in 10 cc. of hot ethylene glycol was added and heated one hour at 100–110°. The mixture was cooled and diluted with 30 cc. of ethanol and the product centrifuged, washed, and dried. Weight was 500 mg. This product after hydrolysis was shown to contain 0.5–1.0% pteroylglutamic acid by bioassay against *S. faecalis* R.

Acknowledgment.—The authors wish to acknowledge the technical assistance of E. Boggiano, and A. Buxó in obtaining ultraviolet absorption data and microbiological assays. Infrared absorption comparisons were made by R. C. Gore, Stamford Research Laboratories, American Cyanamid Company. Microanalyses were done by Louis Brancone and co-workers.

The authors are especially indebted to Dr. J. H. Williams for his constant interest and counsel and for his efforts in coördinating the work performed in the various laboratories.

Summary

A new synthesis of 2-amino-4-hydroxy-6-methylpteridine is described which includes a description of the dihydro form of this compound as an intermediate.

A synthesis of pteroylglutamic acid from 2-amino-4-hydroxy-6-methylpteridine by halogenation and reaction with diethyl N-(*p*-aminobenzoyl) glutamate is described.

PEARL RIVER, NEW YORK RECEIVED JANUARY 24, 1947

(6) Angier, *et al.*, *Science*, **103**, 667 (1946).