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Synthesis of Pteroylglutamic Acid (Liver *L. casei* Factor) and Pteroic Acid. II

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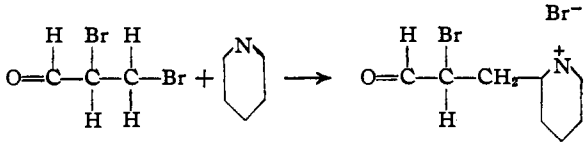
In a previous publication,[5,6] pteroylglutamic acid and pteroic acid were synthesized by reactions involving 2,4,5-triamino-6-hydroxypyrimidine, 2,3-dibromopropionaldehyde and *p*-aminobenzoylglutamic or *p*-aminobenzoic acid. It was felt that preparation of a 6-substituted pteridine capable of alkylating the above amino acids was desirable. Descriptions in the literature suggest the use of quaternary ammonium salts as alkylating agents, such as the procedure of Snyder, Smith and Stewart[7] in alkylating malonic and similar esters with benzyl trimethylammonium salts or gramine to obtain the corresponding C-benzyl malonic ester or substituted esters of β-(3-indole)-propionic acid.

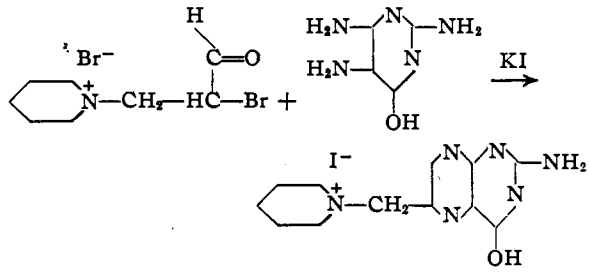
The above workers and others made use of this type of reaction in the preparation of synthetic *dl*-tryptophan[8,9,10]; and the use of simple quaternary salts in the preparation of sulfur[11] and oxygen[12,13,14,15] alkylated derivatives has also been described.

The use of a tertiary amine to alkylate another amine is reported by Howe, *et al.*[10] The effectiveness of pyridine as the tertiary base component of the quaternary ammonium salt was shown by Snyder and Speck.[11]

α,β-Dibromopropionaldehyde was condensed with pyridine to give the intermediate, N-(2-formyl-2-bromoethyl)-pyridinium bromide



and this compound was then condensed with the 2,4,5-triamino-6-hydroxypyrimidine to give the desired N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium bromide for use in alkylation.



Presumably a dihydro derivative was formed which, during the course of the reaction, was converted to the aromatic form. The product was isolated as the less soluble iodide.

Oxidation of the N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide gave the corresponding 2-amino-4-hydroxypteridine-6-carboxylic acid,[16] showing the substituted methyl group to be in the 6- position. No 2-amino-4-hydroxypteridine-7-carboxylic acid was detected.

Pteroylglutamic acid or pteroic acid was then prepared by heating the N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide, *p*-aminobenzoylglutamic acid or *p*-aminobenzoic acid and sodium methylate in anhydrous ethylene glycol. After isolation of the crude material, purification was carried out as described by Waller, *et al.*[6] The crystalline structure, ultraviolet absorption spectra, and bioassay of the synthetic pteroylglutamic acid were identical with those same properties of the liver *L. casei* factor. The properties of the synthetic pteroic acid were identical with those of the pteroic acid prepared by Waller, *et al.*[6]

Experimental

Materials.—The 2,4,5-triamino-6-hydroxypyrimidine, *p*-aminobenzoyl-*l*(+)-glutamic acid and dibromopropionaldehyde were prepared as described in the previous paper in this series.[6]

N-(2-Formyl-2-bromoethyl)-pyridinium Bromide.—To a solution of 21.6 g. (0.1 mole) of freshly distilled 2,3-dibromopropionaldehyde in 100 ml. of anhydrous ether at 0° was added slowly 8.8 g. (0.11 mole) of dry pyridine in 100 ml. of anhydrous ether, with stirring and cooling to keep the temperature of the reaction mixture at 0 to 10°. There was an immediate precipitation of a white product at the start of the addition, and, after allowing the mixture to stand for one or two hours, the hygroscopic solid was filtered, washed with ether and dried in a vacuum desiccator, or extracted with water for use directly in the next step. The crude material melted at 66–69°.

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(5) Angier, *et al.*, *Science*, **103**, 667 (1946).
(6) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948).
(7) Snyder, Smith and Stewart, *ibid.*, **66**, 200 (1944).
(8) Snyder and Smith, *ibid.*, **66**, 350 (1944).
(9) Albertson, Archer and Suter, *ibid.*, **66**, 500 (1944); **67**, 36 (1945).
(10) Howe, Zambito, Snyder and Tishler, *ibid.*, **67**, 38 (1945).
(11) Snyder and Speck, *ibid.*, **61**, 668, 2895 (1939).
(12) Hla Baw, *Quart. J. Indian Chem. Soc.*, **3**, 101 (1926) [*C. A.*, **20**, 3695 (1926)].
(13) Tarbell and Vaughan, THIS JOURNAL, **65**, 231 (1943).
(14) Willstätter, *Ber.*, **35**, 584 (1902).
(15) Griess, *ibid.*, **6**, 585 (1873); **13**, 246 (1880).
(16) Mowat, *et al.*, THIS JOURNAL, **70**, 14, (1947).

Anal. Calcd. for C_8H_9NBr: Br, 27.09. Found: Br, 29.8.

It was recrystallized from absolute 2-propanol, and then melted to a milky liquid at 55–65°, cleared at 80–90°, became partially crystalline again at 105–110°, and melted at 120–125°.

Anal. Calcd.: Br, 27.09. Found: Br, 27.55, 27.59.

N-[(2-Amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium Iodide.—To a solution of 14.1 g. (0.1 mole) of 2,4,5-triamino-6-hydroxypyrimidine in 150 ml. of water and 20 ml. of concd. hydrochloric acid was added the N-(2-formyl-2-bromoethyl)-pyridinium bromide prepared as above in a 0.1-mole run and extracted from the ether with 50 ml. of water. The yellowish-brown solution became almost purple. There was then added 10 g. of potassium iodide, and the solution was allowed to stand overnight. After addition of sodium hydroxide solution to bring the *p*H to 3 to 4, the mixture of brown, amorphous material and lighter crystalline product was filtered off and recrystallized from water, using activated charcoal to decolorize the hot solution. The addition of potassium iodide aided in obtaining more complete crystallization of the less soluble iodide. The purified material consisted of bright yellow, thin plates and rosets of plates.

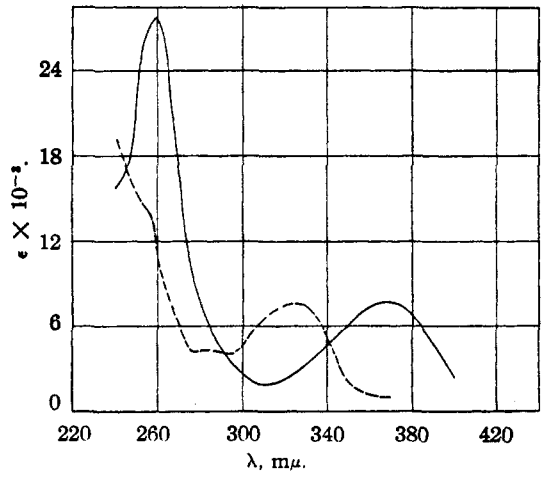

Fig. 1.—Ultraviolet absorption spectra of N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide: ——— 0.1 *N* sodium hydroxide; - - - - - - - 0.1 *N* hydrochloric acid.[a]

[a] ϵ is the molecular extinction coefficient as defined by $I = I_0\ 10^{-\epsilon cl}$ where c is the concentration in moles/liter and l is the cell length in centimeters. Transmittancy (I/I_0) measurements of 10 mg./liter solutions were made in 1-cm. cells at 5 mμ intervals on a Model DU Beckmann Spectrophotometer using a solvent filled cell in the reference position. Additional data were obtained at 2 mμ intervals at maxima, minima and points of inflection.

Anal. Calcd. for $C_{12}H_{11}ON_6I$: N, 22.0; I, 33.2. Found: N, 21.9; I, 32.7.

The ultraviolet absorption curve for this compound is given in Fig. 1.

Oxidation of the above product with hot alkaline permanganate yielded 2-amino-4-hydroxypteridine-6-carboxylic acid.[16] An odor of pyridine was noted during the oxidation step.

Pteroylglutamic Acid.—To a solution of 10.8 g. (0.2 mole) of sodium methylate in 75 ml. of anhydrous ethylene glycol was added 26.6 g. (0.1 mole) of *p*-aminobenzoyl-*l*(+)-glutamic acid and 38.2 g. (0.1 mole) of N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide. A solution of 5.4 g. (0.1 mole) of sodium methylate in 25 ml. of anhydrous ethylene glycol was added, and the resulting solution was heated at 140–145° for three hours. The brown solution obtained was poured into one liter of water at 70°, and acidified to *p*H 3 to 4 with hydrochloric acid. The precipitated product filtered from the cooled solution weighed 9 g., and was shown to contain 25% pteroylglutamic acid by bioassaying with *S. faecalis* R.

Purification of this crude material as described by Waller, *et al.*,[6] gave pteroylglutamic acid showing crystalline structure, ultraviolet absorption spectra and bioassay identical with those same properties of liver *L. casei* factor.

Pteroic Acid.—This was prepared as described under pteroylglutamic acid, substituting *p*-aminobenzoic acid for *p*-aminobenzoyl-*l*(+)-glutamic acid, and decreasing the sodium methylate usage by one mole per mole of N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide.

Purification as described by Waller, *et al.*,[6] gave pteroic acid. The ultraviolet spectra in alkaline solution showed this to be identical with Waller's material.

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Summary

1. N-[(2-Amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide has been prepared by the reaction of N-(2-formyl-2-bromoethyl)-pyridinium bromide with 2,4,5-triamino-6-hydroxypyrimidine.

2. Pteroylglutamic acid and pteroic acid have been prepared by alkylating the appropriate amino acid with N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-pyridinium iodide.

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