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The Synthesis of Pteroylglutamic Acid

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A new approach to the synthesis of pteroylglutamic acid is described. Thus 2-acetamido-4-hydroxy-6-formylpteridine and *p*-aminobenzoylglutamic acid are condensed in the presence of either formic acid or thiocresol to give excellent yields of pteroylglutamic acid derivatives of high purity. All the intermediates of this series are highly crystalline and are easily purified by recrystallization from polar solvents. These intermediates are readily converted to pure pteroylglutamic acid by basic hydrolysis.

Pteroylglutamic acid, an important vitamin of the B complex, has been synthesized previously in different laboratories.[1] It is well known that pterines of this series are usually intractable solids which have limited solubilities in organic solvents and very high decomposition points. Purification of these compounds is therefore very difficult and entails involved techniques. It was the purpose of this investigation to design a new synthetic approach to this important vitamin which could utilize crystalline intermediates, with the concomitant result that relatively high purity pteroylglutamic acid would be obtainable with a minimum of purification. Toward this end, the following synthesis was improvised.

The bromination of 1,3,3-triethoxypropene (I)[2] in an inert solvent yielded the bromo-ether II which was selectively hydrolyzed in aqueous bicarbonate to 2-bromo-3,3-diethoxypropionaldehyde (III) in a 60% yield. This aldehyde is a viscous colorless oil, easily distillable *in vacuo* and stable under anhydrous conditions.

The condensation of 2,4,5-triamino-6-hydroxypyrimidine (IV), and the bromo-aldehyde III, was readily carried out in an aqueous bicarbonate medium to give the desired 2-amino-4-hydroxy-6-diethoxymethylpteridine, which was purified by crystallization of the sodium salt. In order to prove that V was the desired isomer and not the 2-amino-4-hydroxy-7-diethoxypteridine, it was oxidized in alkaline permanganate to the 2-amino-4-hydroxy-6-carboxypteridine. Paper strip chromatography, as described by Weygand,[3] demonstrated the presence of only the desired isomer.

Upon acetylation of V in acetic anhydride at 100°, an excellent yield of crystalline 2-acetamido-4-hydroxy-6-diethoxymethylpteridine (VI) was obtained. This white crystalline compound was easily purified by recrystallization from either alcohol or dioxane. Selective cleavage of the acetal group was achieved without hydrolysis of the acyl function by dissolving VI in 88% formic acid and allowing it to stand at room temperature for four hours. As the reaction proceeded, the formic acid salt of the pteride-aldehyde VII crystallized from the solution.

The reductive condensation[4] of 2-amino-4-hydroxy-6-formylpteridine and *p*-aminobenzoylglutamic acid, or of the corresponding Schiff base by catalytic means, is a poor reaction. Apparently, the pteridine ring is very susceptible to reduction and many by-products are thereby formed. It was found that aryl thiols[5] were excellent selective reducing agents for this series. Yields were in the range of 54–60% of theoretical of N^2-acetylpteroylglutamic acid, which was 83–85% pure This crystalline material was easily purified by recrystallization from water to give an analytically pure compound. In contrast, the unacylated aldehyde, 2-amino-4-hydroxy-6-formylpteridine, gave practically no conversion to the pteroylglutamic acid under the same reaction conditions.

A study of this reaction revealed that maximum yields were obtained when a fivefold excess of the aryl-thiol was used. Further it was found that the aliphatic mercaptans such as thioacetic acid, thioglycolic acid, *t*-butyl mercaptan, as well as thiourea gave lower conversions to pteroylglutamic acid than the aryl thiols. The effects of acid catalysis, such as toluenesulfonic acid, and peroxides were investigated. No appreciable influence on the conversion to N^2-acetylpteroylglutamic acid was observed.

It has been found that formic acid[6] is a potent reducing agent for this series. Thus formic acid reduces VII and *p*-aminobenzoylglutamic acid to the N^2-acetyl-N^{10}-formyl-glutamic acid in a yield comparable to the thiol reduction. This reaction proceeds at room temperature but goes to a maximum yield in a minimum of time at 68°. Anhydrous

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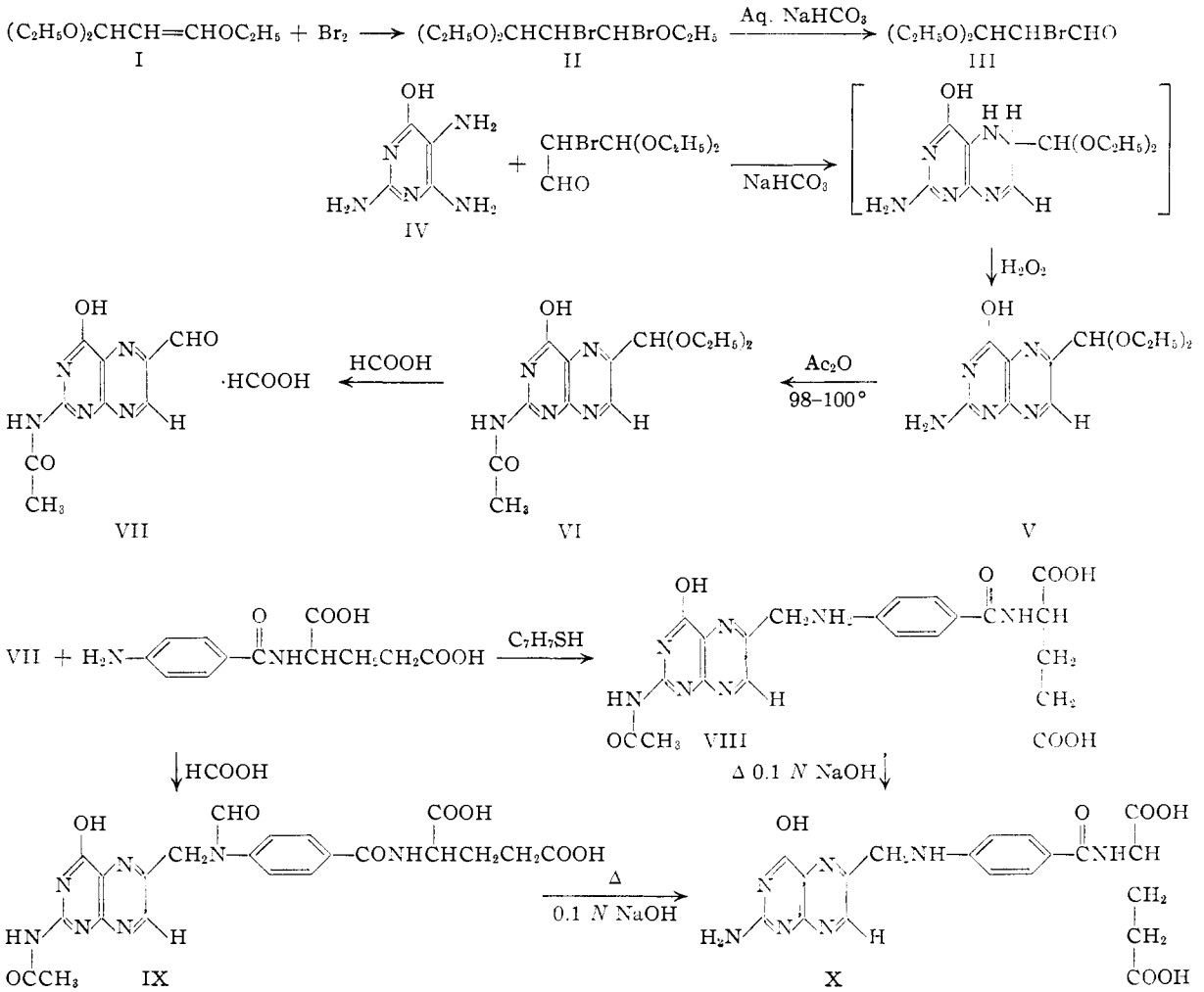

formic acid is necessary in order to obtain maximum conversion to IX. N^2-Acetyl-N^{10}-formylpteroylglutamic acid is a crystalline pale yellow compound which has a marked water solubility and is purified from this medium to an analytically pure product. Upon hydrolysis in hot 0.1 *N* sodium hydroxide, IX is converted to pteroylglutamic acid.

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Experimental

1-Bromo-2,2-diethoxypropionaldehyde (III).—To a solution of 425 g. (2.44 moles) of 1,3,3-triethoxypropene-1 in 1050 ml. of anhydrous ether was added with stirring at 0–10° over a period of 30 minutes, 355 g. (2.22 moles) of bromine or until a slight orange color of bromine remained. The ether solution was concentrated *in vacuo* with a maximum bath temperature of 30°. The yellow residue was then added with stirring to a mixture of 323 g. of sodium bicarbonate, 765 ml. of dioxane and 256 ml. of water, maintaining the temperature between 5–10° by external cooling in 40 minutes. Stirring was then maintained for an additional two hours at 0–5°. The mixture was extracted with ether and the organic layer was dried over anhydrous sodium sulfate. This was filtered and concentrated *in vacuo*. The residue was fractionated using a Vigreux column to yield 330 g. (66%) of colorless material, b.p. 65–70° (4 mm.), $n^{25}D$ 1.4500.

Anal. Calcd. for $C_7H_{13}O_3Br$: C, 37.37; H, 5.81; Br, 35.50. Found: C, 37.59; H, 5.80; Br, 35.7.

2-Amino-4-hydroxy-6-diethoxymethylpteridine (V).—Into a solution of 214 g. of $NaHCO_3$ in 4.2 liters of water was added with agitation 150 g. (0.705 mole) of 2,4,5-triamino-6-hydroxypyrimidine hydrochloride (IV). After the evolution of CO_2 had ceased, 143 g. (0.635 mole) of the bromoaldehyde III was added over a 30-minute period, keeping the temperature at 25–30°. Good agitation was maintained throughout the reaction. The mixture was left to age for one hour at 20–25° and then oxidized with 150 g. of 30% H_2O_2 in 320 ml. of H_2O. The temperature of this oxidation was kept at 25–30°. Foaming occurred and some ether was added to break up the foam. After all the peroxide was added, the mixture, which had taken on a black color, was stirred for 1.5 hr. at 25–30° and again treated with 75 ml. of 30% H_2O_2 in 150 ml. of H_2O. The reaction was now left to stand at 25° for 18 hours, filtered, washed with water, acetone and dried at 80°.

The crude pteride was purified by dissolving in a solution of 237 ml. of 2.5 *N* NaOH in 1230 ml. of water and filtered from some red insoluble material. Pellets of NaOH (156 g.) were added to the filtrate and the solution stirred until all the base had dissolved. The temperature rose to 50° and within a short period a copious tan precipitate of the sodium salt separated. After cooling to 5° for 2 hours the mixture was filtered and sucked as dry as possible. The solid was dissolved in 1180 ml. of water and slowly acidified with 36% acetic acid until a *p*H of 3 was obtained. The resulting cream-colored precipitate was aged at 5° for 3 hours, filtered, washed with water and dried at 80° in a vacuum of 50 mm. The yield was 118 g. λ_{max} (0.1 *N* NaOH) 2550, 3620 (*E* 1% 941; 288); λ_{max} (0.1 *N* HCl) 3180 (*E* 1% 340).

Anal. Calcd. for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.66; N, 26.4. Found: C, 50.05; H, 5.65; N, 26.40.

In order to demonstrate that V was the desired isomer, 2 g. of V was dissolved in 10 ml. of 2.5 *N* KOH and this was heated with 80 ml. of a saturated $KMnO_4$ solution at 90° for one hour. The excess permanganate was decolorized with sodium sulfite. The solution was filtered from the insoluble MnO_2 and the filtrate acidified with acetic acid. The tan precipitate was centrifuged, separated from the supernatant liquid and washed with water and acetone. The solid, after drying, weighed 1.3 g. Paper strip chromatography using Whatman #1 paper as described by Weygand[3] showed an R_f 0.39–0.40 with a blue fluorescence.

2-Acetamido-4-hydroxy-6-diethoxymethylpteridine (VI).—A mixture of 439 g. (1.66 moles) of V suspended in 3073 ml. of acetic anhydride with stirring was heated at 100° for 4 hours. During this time most of the solid dissolved and a small amount of red insoluble material remained. This was removed by a hot filtration at 100°. The yellow filtrate was cooled to 25° slowly, and finally in ice to 5°. The copious precipitate was washed with cold acetic anhydride, benzene and finally dried at 100° at 10–15 mm. The dried solid was recrystallized from 3600 ml. of absolute ethanol using 40 g. of Norite for decolorization. On slow cooling the filtrate deposited a copious precipitate. The mixture was filtered, washed with two 100-ml. portions of cold ethanol and dried at 100° at 30–50 mm.

The Norite cake was refluxed with ethanol filtrate from above for one hour and filtered. Upon concentration of the filtrate to a volume of 300 ml. and cooling slowly, an additional 14 g. of product was obtained. The total yield was 432 g. (85%) of m.p. 197–200° of white crystalline solid, λ_{max} 2570, 3500 (*E* 1% 835, 240) in 0.1 *N* NaOH.

Anal. Calcd. for $C_{13}H_{17}N_5O_4$: C, 50.81; H, 5.5; N, 22.8. Found: C, 50.81; H, 5.45; N, 23.09.

2-Acetamido-4-hydroxy-6-formylpteridine (VII).—Six hundred grams of the 2-acetamido-4-hydroxy-6-diethoxypteridine (VI) was added with stirring to 3000 ml. of 88% formic acid at 25°. All the solid dissolved and after 10 minutes a white crystalline precipitate of the formic acid salt of the pteridinealdehyde VII separated. Agitation was maintained at 25° for 2 hours and the mixture was then cooled to 5° for 3 hours. The precipitate was filtered, washed with cold formic acid, ether and dried at 25° in a vacuum of 50–55 mm. The yield of white crystalline solid was 494 g.

From the acid mother liquors an additional 26 g. of product was obtained after dilution with ether. The total yield was 520 g. (95%), λ_{max} 2550, 3500 (*E* 1% 816, 297) in 0.1 *N* NaOH.

For analysis, 5 g. of above product was recrystallized from 50 ml. of dimethylformamide by heating to 120° and slow cooling. In the course of the recrystallization a mole of formic acid was removed, λ_{max} 2550, 3500 (*E* 1% 961, 342).

Anal. Calcd. for $C_9H_7N_5O_3$: C, 46.40; H, 3.0; N, 30.1. Found: C, 46.57; H, 2.98; N, 29.6.

N^2-Acetylpteroylglutamic Acid (VIII).—A mixture of 6.7 g. (0.0288 mole) of aldehyde (VII) freed of formic acid, 10 g. (0.0376 mole) of *p*-aminobenzoylglutamic acid, 25 g. (0.2 mole) of *p*-thiocresol and 250 ml. of redistilled methyl Cellosolve was heated in a nitrogen atmosphere, with agitation, to the boiling point of the solution and maintained at reflux for 3.5 hours. The hot gelatinous reaction mixture was added to 3500 ml. of hot water (80–90°) and the water was distilled until no more oil droplets of unreacted thiocresol were detected in the distillate. This required about 500 ml. of distillate. The hot residue was now filtered from a small amount of amorphous solid and allowed to cool slowly to 25° and finally cooled to 5° for 3 hours and then filtered. The yellow crystalline acetylpteroylglutamic acid was washed with cold water, acetone and dried at 80°. The yield was 7.8 g. of solid which assayed as 83–85% *vs. Lactobacillus casei.*[7] This represents a 54% yield.

For analysis 2 g. of solid was recrystallized from 500 ml. of water. On cooling, beautiful yellow crystals separated which were filtered, washed with water, acetone and dried at 120° (5 mm.) pressure.

Anal. Calcd. for $C_{21}H_{21}N_7O_7$: C, 52.17; H, 4.37; N, 20.3. Found: C, 52.53; H, 4.25; N, 20.7.

N^2-Acetyl-N^{10}-formylpteroylglutamic Acid (IX).—To a solution of 800 ml. of 98% formic which had been fortified with 90 g. of acetic anhydride was added 93 g. (0.33 mole) of 2-acetamido-6-formylpteridine (VII) and 186 g. (0.70 mole) of *p*-aminobenzoylglutamic acid. The mixture was stirred, under anhydrous conditions, and heated to 68° and kept at this temperature for one hour. During this period carbon monoxide was liberated and the solution took on a brown color. The mixture was cooled to 25° and added to 5 liters of anhydrous ether with stirring. After stirring for one hour the mixture was filtered under anhydrous conditions. The solid was well washed with ether and then dried *in vacuo* at 30°. The yield was 258 g. which assayed as 37% pure by *Lactobacillus casei.*[7] This represents a 56% yield.

In order to purify the above material, 5 g. was dissolved in 175 ml. of refluxing ethanol. After cooling to 40° in 45 minutes, a slight amount of brown solid separated which was filtered. The filtrate was concentrated to a volume of 130 ml. *in vacuo* and left to stand for 18 hours. At the end of this period an orange precipitate settled which was filtered, washed with alcohol and dried at 80°. The yield was 1.8 g. of orange solid which assayed as 85% pure by *Lactobacillus casei.*[7]

In order to obtain analytically pure material, 10 g. of the above solid was extracted with 200 ml. of boiling water. Upon immediate chilling the extract in ice, solid separated which was filtered with the aid of Super-Cel. The Super-Cel cake was again extracted with four 200-ml. portions of boiling water, cooled and filtered as above. On cooling the combined filtrates for 18 hours at 5°, pale-yellow crystals were deposited. After filtration and washing with water and isopropyl alcohol, the solid was dried at 80°. A yield of 7.5 g. of N^2-acetyl-N^{10}-formylpteroylglutamic acid was obtained which assayed as 98–100% pure against *Lactobacillus casei.*[7]

Anal. Calcd. for $C_{22}H_{21}N_7O_8$: C, 51.44; H, 4.13; N, 19.12. Found: C, 51.44; H, 4.22; N, 19.02.

Pteroylglutamic Acid (X). (A).—A solution of 3 g. of N^2-acetylpteroylglutamic acid (85% activity) in 1500 ml. of 0.1 *N* NaOH was heated in a nitrogen atmosphere at 90° for 30 minutes. The solution was treated with 1 g. of Norite and stirred for 30 minutes and filtered. The yellowish filtrate was heated to 90° and acidified to a *p*H of 3 with concd. hydrochloric acid and left to stand at room temperature for 18 hours after seeding with some pteroylglutamic acid. At the end of this period a yellow crystalline precipitate had settled. After chilling to 5° the solution was filtered, washed with water, acetone and dried at 80°. The yield was 2.6 g. This material was 98–100% active against *Lactobacillus casei.*

(B).—A solution of 3 g. of N^2-acetyl-N^{10}-formylpteroylglutamic acid (85% activity) in 1500 cc. of 0.1 *N* NaOH was hydrolyzed as in "A." A yield of 2.2 g. of pteroylglutamic acid was obtained which assayed as 98–100% active against *Lactobacillus casei.*

Anal. Calcd. for $C_{19}H_{19}N_7O_6$: C, 51.72; H, 4.34; N, 22.22. Found: C, 51.97; H, 4.64; N, 21.82.

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(7) For microbiological assay 20 mg. of the sample was first hydrolyzed in 10 cc. of 0.1 *N* NaOH at 90–100° for 30 minutes, neutralized and then diluted to the required volume.