flux for three hours on a steam-bath. After standing at room temperature overnight the crude cream-colored triazine (9.35 g., 51%) was collected and washed with ethyl acetate. The substance was dissolved in cold *N* potassium hydroxide and the solution was treated with charcoal, filtered and acidified with acetic acid, when the triazine was precipitated. Recrystallization from ethoxyethanol gave the pure triazine as small colorless needles which melted at 265°, with darkening and gassing below the melting point.[10]

Anal. Calcd. for $C_{10}H_{10}N_4S$: C, 55.0; H, 4.6; N, 25.7; S, 14.7. Found: C, 55.3; H, 4.7; N, 25.4; S, 14.3.

The picrate separated from aqueous alcohol in yellow needles, m.p. 187–188°.[10]

Anal. Calcd. for $C_{10}H_{10}N_4S \cdot C_6H_3O_7N_3$: C, 42.9; H, 2.9; N, 21.9; S, 7.2. Found: C, 42.4; H, 3.1; N, 21.4; S, 7.4.

2-Amino-4-*p*-chlorobenzyl-6-mercapto-1,3,5-triazine.—This compound was prepared in a manner analogous to that described above for the unsubstituted compound. The crude triazine was obtained in 58% yield. It was purified by solution in dilute ammonium hydroxide followed by precipitation with acetic acid, and finally by recrystallization from ethoxyethanol. It formed colorless prisms, m.p. 268–269° (dec.).

Anal. Calcd. for $C_{10}H_9N_4ClS$: C, 47.6; H, 3.6; N, 22.2. Found: C, 47.6; H, 3.6; N, 22.2.

The picrate separated from ethanol in clusters of tiny yellow needles, m.p. 196–197°.

Anal. Calcd. for $C_{10}H_9N_4ClS \cdot C_6H_3O_7N_3$: N, 20.4; S, 6.7. Found: N, 20.3; S, 6.9.

2-Amino-4-benzyl-1,3,5-triazine.—2-Amino-4-benzyl-6-mercapto-1,3,5-triazine (2.0 g.) was treated with Raney nickel (2.0 g., prepared without allowing the temperature to rise above 50°) in boiling ethanol (100 ml.), two further portions of Raney nickel (each of 2 g.) being added after one-half and 1.5 hours, respectively. After refluxing for two hours, the suspension was filtered and the nickel washed with hot ethanol. The combined ethanolic solutions were evaporated *in vacuo* and the greenish residue was shaken with a slight excess of cold *N* hydrochloric acid. The solution, freed from a small amount of insoluble material, was basified with an excess of *N* sodium hydroxide solution and extracted with ether (twice with 250 ml.). The colorless residue (0.55 g., 32%) obtained on evaporation of the ether gave, on recrystallization from benzene, colorless flattened needles of 2-amino-4-benzyl-1,3,5-triazine, m.p. 139–140°, identical with the product, m.p. 138.5–139.5°, obtained from formylphenylacetonitrile and guanidine[3] or from formylguanidine and phenylacetonitrile.

Anal. Calcd. for $C_{10}H_{10}N_4$: C, 64.5; H, 5.4; N, 30.1. Found: C, 64.2; H, 5.3; N, 30.1.

2-Amino-4-*p*-chlorobenzyl-1,3,5-triazine.—2-Amino-4-*p*-chlorobenzyl-1,3,5-triazine was prepared by desulfurization of the corresponding 6-mercapto compound in a manner analogous to that described above. The product was crystallized from *n*-butanol to give colorless flattened prisms, m.p. 205–206° which did not depress the melting point (205–206°) of the product obtained from α-formyl-*p*-chlorophenylacetonitrile and guanidine.[3]

Anal. Calcd. for $C_{10}H_9N_4Cl$; N, 25.4. Found: N, 25.0.

2,4-Diamino-6-*p*-chlorobenzyl-1,3,5-triazine.—This compound was prepared by the method of Ostrogovich and Gheorghiu.[11]

Dicyandiamide (4 g.) and *p*-chlorophenylacetonitrile (8 cc.) in ethanol (10 ml.) were heated in an open flask at 180–200° for two hours. After cooling the residue was washed with ether and then suspended in sodium hydroxide solution. After filtration the solid was recrystallized from ethanol. It formed needles, m.p. 252°.

Anal. Calcd. for $C_{10}H_{10}N_5Cl$: C, 51.0; H, 4.3; N, 29.9. Found: C, 51.2; H, 4.3; N, 30.4.

(10) A. Ostrogovich and V. Galea (ref. 6) who gave scanty details record melting points of 270–271° and 187–188° for the triazine and picrate, respectively.

(11) A. Ostrogovich and G. Gheorghiu, *Gazz. chim. ital.*, **60**, 648 (1930).

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[Contribution from the Lederle Laboratories Division, American Cyanamid Company]

7-Isomer of Pteroylglutamic Acid

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The bromination of 2-amino-4-hydroxy-7-methylpteridine in 48% hydrobromic acid gave a 7-bromomethyl or a 7-dibromomethyl derivative depending upon the quantity of bromine used. From the monobrominated compound was prepared the 7-isomer of pteroylglutamic acid. The instability of this 7-isomer accounts for its absence in purified synthetic pteroylglutamic acid.

In previous communications on the synthesis of pteroylamino acids[1,2] only the 6-isomers have been reported and in the purified compounds[1,2]: the absence of the 7-isomer was proven by oxidation to give only the 2-amino-4-hydroxy-6-pteridinecarboxylic acid. The 7-isomer of pteroic acid was reported to be formed by treating two molecular proportions of *p*-aminobenzoic acid with one of reductone and then treating the product with 2,4,5-triamino-6-hydroxypyrimidine.[3] However, Forrest and Walker did not characterize their product beyond the oxidation of the crude to 2-amino-4-hydroxy-7-pteridinecarboxylic acid.

Disclosed herein is the synthesis of the 7-isomer of pteroylglutamic acid, "7-pteroylglutamic acid" (IV) N-[4-{[(2-amino-4-hydroxy-7-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid.

The bromination[4] of 2-amino-4-hydroxy-7-methylpteridine[5] (I) in 48% hydrobromic acid gave 2-amino-4-hydroxy-7-bromomethylpteridine (II) when one molecular proportion of bromine was used and 2-amino-4-hydroxy-7-dibromomethylpteridine (III) when an excess of bromine was used. The monoacetyl derivative of the latter pterin was prepared by heating at refluxing temperature in acetic anhydride.

The ultraviolet absorption data for the 7-bromomethylpteridine II and 7-dibromomethylpteridine

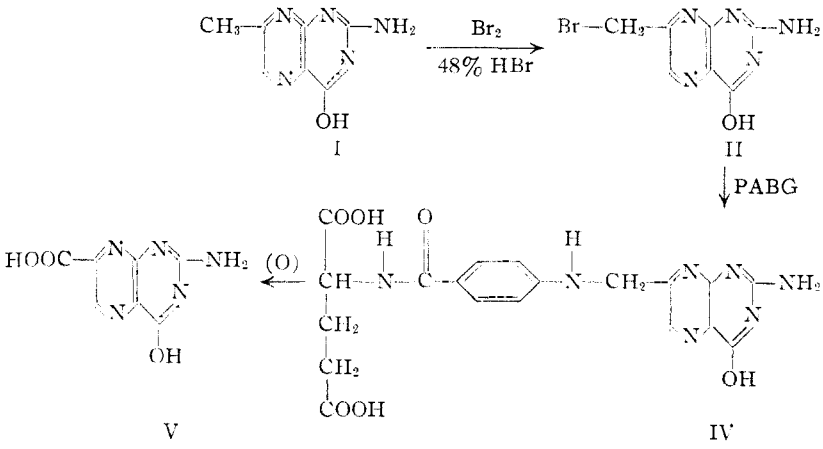
(1) C. W. Waller, *et al.*, This Journal, **70**, 19 (1948).

(2) (a) R. B. Angier, *et al.*, *ibid.*, **70**, 25 (1948); (b) D. B. Cosulich and J. M. Smith, *ibid.*, **70**, 1922 (1948).

(3) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 79 2002 (1949).

(4) J. H. Boothe, *et al.*, This Journal, **70**, 27 (1948); C. W. Waller, *et al.*, *ibid.*, **72**, 4630 (1950).

(5) J. H. Mowat, *et al.*, *ibid.*, **70**, 14 (1948); R. B. Angier, *et al.* *ibid.*, **70**, 3029 (1948).



III are represented by Figs. 1 and 2, respectively. The data for the second maximum in 0.1 *N* sodium hydroxide solution shows a bathochromic shift of about 10 mμ for each added bromine atom over that of the 2-amino-4-hydroxy-7-methylpteridine.[5]

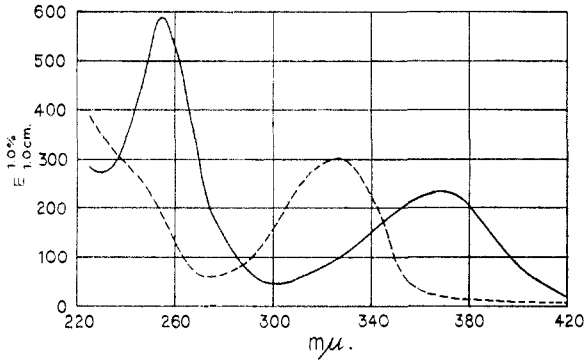


Fig. 1.—Ultraviolet absorption spectra of 2-amino-4-hydroxy-7-bromomethylpteridine hydrobromide: ——, 0.1 *N* sodium hydroxide; - - - -, 0.1 *N* hydrochloric acid.

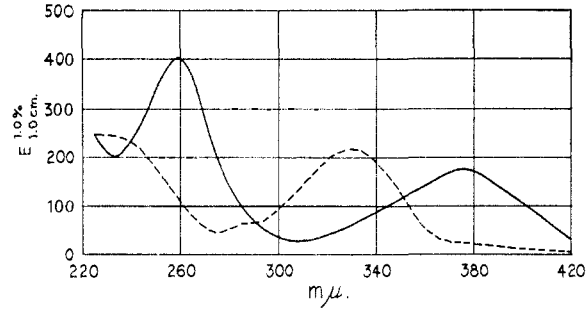


Fig. 2.—Ultraviolet absorption spectra of 2-amino-4-hydroxy-7-dibromomethylpteridine hydrobromide: ——, 0.1 *N* sodium hydroxide; - - - -, 0.1 *N* hydrochloric acid.

The condensation of II with *p*-aminobenzoyl-L(+)-glutamic acid (PABG) gave 7-pteroylglutamic acid (IV). The crude IV was purified by crystallization from dilute hydrochloric acid.[6] This compound was very unstable both in the solid state as the free acid or in an alkaline solution. It was oxidized very readily at room temperature by potassium permanganate to 2-amino-4-hydroxy-7-pteridinecarboxylic acid.[5] The ultraviolet absorption spectra, crystalline structure and solubilities of 7-pteroylglutamic acid are almost identical with pteroylglutamic acid.

When the –methylaminobenzoylglutamic acid group is attached to the pteridine ring in the 7-position an unstable compound results as shown by 7-pteroylglutamic acid, 6-carboxy-7-pteroylglutamic acid[7] and 6-methyl-7-pteroylglutamic acid.[8] The instability of the 7-isomers furnishes the explanation for their complete absence in the purified 6-isomers.

Experimental

2-Amino-4-hydroxy-7-bromomethylpteridine (II).—To a vigorously stirred solution of 10 g. of 2-amino-4-hydroxy-7-methylpteridine (I) dissolved by heating on a steam-bath in 300 cc. of 48% hydrobromic acid was added dropwise 10 g. of bromine in 30 cc. of 48% hydrobromic acid. The solution was heated on the steam-bath for 20 minutes after all the bromine had been added, and then concentrated under vacuum to 50 cc. This concentrate was cooled overnight at −5°, filtered, and evaporated under vacuum to dryness. The residue was ground in 300 cc. of water, filtered, washed with water, alcohol and ether and dried, yield 11 g. of crude. The crude was purified by recrystallization from 48% hydrobromic acid.

Anal. Calcd. for $C_7H_6N_5OBr \cdot HBr$: C, 24.95; H, 2.08; N, 20.78; Br, 47.32. Found: C, 25.34; H, 2.38; N, 20.26; Br, 47.37.

2-Amino-4-hydroxy-7-dibromomethylpteridine (III).—To a solution of 2 g. of I dissolved in 40 cc. of boiling 48% hydrobromic acid was added with stirring 4 g. of bromine. This mixture was heated under reflux condenser for 45 minutes and then concentrated under vacuum to remove excess bromine. The white crystalline product, which separated after cooling overnight, was collected and washed with 48% hydrobromic acid. This product was suspended in water containing a slight excess of pyridine. The white crystalline material changed to a yellow powder. This yellow powder was collected, washed, and dried; yield 2.3 g.

Anal. Calcd. for $C_7H_5ON_5Br_2$: C, 25.10; H, 1.50; N, 20.91; Br, 47.71. Found: C, 25.33; H, 0.76; N, 20.7; Br, 47.6.

A sample of III when dissolved in 48% hydrobromic acid immediately crystallized in long white needles. This product was collected, washed with 48% hydrobromic acid and ether and dried.

Anal. Calcd. for $C_7H_5ON_5Br_2 \cdot HBr$: C, 20.21; H, 1.45; N, 16.84. Found: C, 20.41; H, 2.18; N, 16.53.

The bromine atoms were proved to be in the methyl group by two experiments. A sample of III was reduced with hydriodic acid in glacial acetic acid to give a 60% yield of pure 7-methylpterin (I). Secondly, the oxidation of III with alkaline permanganate gave an 85% yield of the 7-carboxypterin (V).

2-Acetylamino-4-hydroxy-7-dibromomethylpteridine.—A solution of 1 g. of 2-amino-4-hydroxy-7-dibromomethylpteridine hydrobromide in 25 cc. of acetic anhydride was refluxed for 20 minutes. The solution was clarified with charcoal and evaporated to dryness. The residue was crystallized from a methyl cellosolve–water mixture.

Anal. Calcd. for $C_9H_7N_5O_2Br_2$: C, 28.6; H, 1.87; N, 18.5; Br, 42.3. Found: C, 29.2; H, 2.09; N, 18.9; Br, 42.3.

N-[4-{[(2-Amino-4-hydroxy-7-pteridyl)-methyl]-amino}-benzoyl]-glutamic Acid, or "7-Pteroylglutamic Acid" (IV).—Ten grams of II was added in portions to a solution of 48 g. of PABG in 1240 cc. of water maintaining the *p*H at 10 to

(6) C. W. Waller, U.S. Patent 2,474,022 (1949).

(7) C. W. Waller, *et al.*, THIS JOURNAL, **74**, in press (1952).

(8) J. H. Boothe, *et al.*, *ibid.*, **74**, 5407 (1951).

11 by adding a dilute solution of sodium hydroxide. When the *p*H became constant (about one hour) the solution was heated quickly to 80°, acidified to *p*H 2–3, cooled and filtered. The air-dried product (13 g.) assayed chemically[9] for 56.2% pteroylglutamic acid, bioassay using *S. faecalis* R. 0.064%.

The crude IV was purified by dissolving in 6 *N* hydrochloric acid, diluting to 1 *N*, warming to 60–70°, clarifying with charcoal and cooling. The procedure was repeated several times to obtain a pure sample. This purified material decomposed immediately on heating at 100° in vacuum or at room temperature for 2 to 3 days. PABG was liberated on decomposition. For analysis a sample was filtered from the 1 *N* hydrochloric acid (where it is stable) washed with water, alcohol and ether, dried at room temperature for 20 minutes under high vacuum and then analyzed immediately.

Anal. Calcd. for $C_{19}H_{19}O_6N_5 \cdot 2H_2O$: C, 47.78; H, 4.85; N, 20.53. Found: C, 47.45; H, 4.99; N, 20.37.

One gram of IV was completely oxidized with alkaline permanganate at room temperature in 10 minutes. The product was purified through its insoluble magnesium salt and its slightly soluble sodium salt. The final product was isolated from dilute hydrochloric acid; yield 0.3 g. Its ultraviolet absorption spectrum was identical with that of V.

The chemical assay for purified IV showed 1.4% free PABG and 90% IV. A 2 *N* hydrochloric acid solution of IV was stable for at least 10 days. The residue from a sodium bicarbonate solution of IV which had been frozen and dried under high vacuum was stable at room temperature for at least 10 days. A sample when isolated from 1 *N* hydrochloric acid solution decomposed in 2 days at room temperature to 51.9% of free PABG and 1.16% of IV. A 0.1 *N* sodium hydroxide solution of IV decomposed in a few hours.

The 7-pteroylglutamic acid was not found to be a growth stimulant for *S. faecalis* R. nor antagonistic to pteroylglutamic acid using this same organism.

The ultraviolet absorption spectrum for IV was identical with that for pteroylglutamic acid.[1]

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(9) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).

[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

7-Methylpteroylglutamic Acid and Some Related Compounds

BY J. H. BOOTHE, J. H. MOWAT, C. W. WALLER, R. B. ANGIER, J. SEMB AND A. L. GAZZOLA

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Three new analogs of pteroylglutamic acid have been prepared, namely, 7-methylpteroylglutamic acid, 7,10-dimethylpteroylglutamic acid and 6-methyl-7-pteroylglutamic acid. These compounds were prepared by treating the corresponding bromomethylpteridine with the requisite *p*-aminobenzoylglutamic acid derivative, and their structures were proved by degradation to known compounds. The first two compounds are biologically active as antagonists of pteroylglutamic acid while the latter compound is inactive as either an antagonist or growth stimulator.

A number of analogs of pteroylglutamic acid have been described in the literature, many of which are antagonists of pteroylglutamic acid in various biological systems. The preparation and properties of pteroylaspartic acid have been described.[1] A derivative of pteroylglutamic acid in which the 4-hydroxyl group is replaced by an amino group has been shown to be a powerful antagonist of pteroylglutamic acid[2a] as has 4-aminopteroylaspartic acid.[2b] Some of the 2,4-diaminopteridines also show some antagonist activity.[3] Alkyl derivatives of pteroylglutamic acid have been prepared including various N^{10}-alkyl analogs[4] as well as C^9-methyl derivatives.[5] The amides of *p*-aminobenzoylglutamic acid and quinoxaline-2-carboxylic acid, xanthopterincarboxylic acid and isoxanthopterincarboxylic acid have been reported[6] to be inhibitors of pteroylglutamic acid. Dibromobutyraldehyde has been substituted for dibromopropionaldehyde and *d*(−)-glutamic acid for *l*(+)-glutamic acid in one of the syntheses of pteroylglutamic acid[7] to yield a product which is an antagonist for pteroylglutamic acid.[8] This product was an impure mixture and the purification or proof of structure has not been reported. However, this crude material was designated "7-methylfolic acid." This same material was also prepared using *l*(+)-glutamic acid and its biochemical properties have been published.[9] The inhibition ratio of this crude antagonist is 20–30 as compared to 7-methylpteroylglutamic which is 252 as indicated later in this paper and 9-methylpteroylglutamic acid[5] which is 2000. Since the inhibition activity of this crude material is approximately 10 times that of the 7-methyl compound and 100 times that of 9-methyl derivative, it is obvious that some other component of this crude material is responsible for its potent antagonist activity.

This communication deals with the preparation, purification and proof of structure of 7-methylpteroylglutamic acid, 6-methyl-7-pteroylglutamic acid and 7,10-dimethylpteroylglutamic acid.

The first attempt to prepare 7-methylpteroylglutamic acid yielded the isomeric 6-methyl-7-pteroylglutamic acid exclusively. This was done through bromination of 2-amino-4-hydroxy-6,7-dimethylpteridine,[10] using one mole of bromine in a manner similar to that used for the preparation of

(1) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **170**, 323 (1947).

(2) (a) D. R. Seeger, J. M. Smith and M. E. Hultquist, THIS JOURNAL, **69**, 2567 (1947); (b) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **180**, 857 (1949).

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(5) M. E. Hultquist, *et al.*, *ibid.*, **71**, 621 (1949).

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(8) G. J. Martin, L. Tolman and J. Moss, *Arch. Biochem.*, **12**, 318 (1947); *Science*, **106**, 168 (1947).

(9) A. L. Franklin, *et al.*, *J. Biol. Chem.*, **169**, 427 (1947).

(10) F. Sachs and G. Meyerheim, *Ber.*, **41**, 3965 (1908).