[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Analogs of Pteroylglutamic Acid. III. 4-Amino Derivatives

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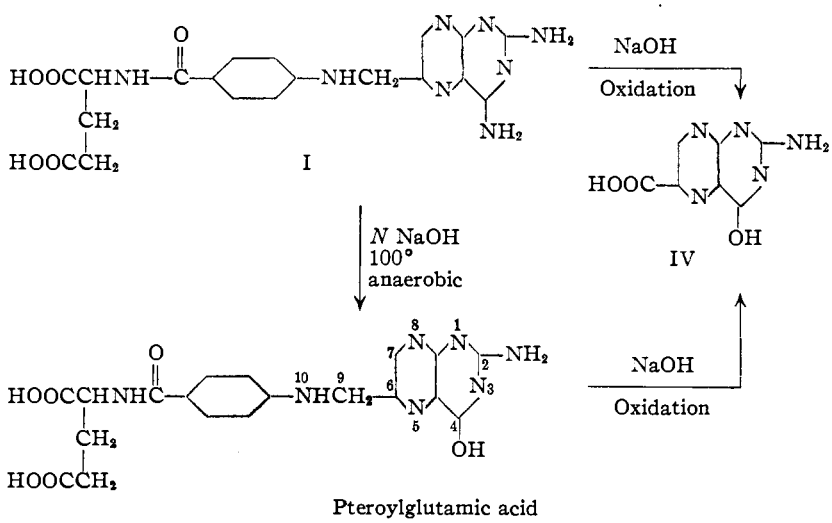
Earlier papers of this series described N^{10}-alkyl[1a] and 9-methyl[1b] analogs of pteroylglutamic acid which exhibited antagonistic action against the latter in the growth of *Streptococcus faecalis* R. The present paper is concerned with derivatives in which the 4-hydroxyl of the pteridine nucleus is replaced by the amino group. The synthesis of the 4-amino analog of pteroylglutamic acid was announced in a preliminary communication[2a] and subsequently a number of references have been made to this compound and its biological properties.[2b-h] The 4-amino derivatives are powerful antagonists for pteroylglutamic acid.

The synthesis of N-[4-{[(2,4-diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid (I) ("Aminopterin") was accomplished by the method of Waller, *et al.*,[3a] from 2,4,5,6-tetraminopyrimidine sulfate,[4a] 2,3-dibromopropionaldehyde, and *p*-aminobenzoyl-l(+)-glutamic acid, and also by the method of Hultquist and Dreisbach,[3b] using 1,1,3-tribromoacetone.[4b] It was purified in a manner similar to that described for pteroylglutamic acid and its derivatives.

4-{N-[(2,4-Diamino-6-pteridyl)-methyl]-N-methylamino}-benzoic acid (II) and N-[4-{N-[(2,4-diamino-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid (III) ("A-Methopterin") were prepared in like manner[3a] using *p*-(N-methylamino)-benzoic and *p*-(N-methylamino)-benzoylglutamic acids[1] in place of *p*-aminobenzoylglutamic acid.

The degradation reactions to which the 4-amino derivatives were submitted were essentially the same methods used with pteroylglutamic acid.[5] No degradation products containing the 2,4-diaminopteridine nucleus were isolated. Evidently the 4-amino group is very readily converted to hydroxyl under the conditions of these experiments. Alkaline permanganate oxidation of I, for example, yielded 2-amino-4-hydroxypteridine-6-carboxylic acid (IV) which has been fully characterized.[6] This same degradation product (IV) was also isolated from the similar treatment of 4{N-[(2,4-diamino-6-pteridyl)-methyl]-N-methylamino}-benzoic acid (II). These reactions located the attachment of the side chain to the pteridine nucleus in the 6-position.



Pteroylglutamic acid

Alkaline aerobic treatment of I resulted in cleavage at the methylene bridge. However, under the same conditions (II) was converted to 4-{N-[2-amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoic acid (V) which had previously been shown to be stable under alkaline aerobic treatment.[1] Similar treatment of III resulted in N-[4-{N-[2-amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid (XI)

The relative stability of the methylene linkage of pteroylglutamic acid in alkaline solution in the absence of oxygen[5] coupled with the ease of hydrolysis of the 4-amino group as indicated above suggested the possibility of converting the 4-amino derivative (I) to pteroylglutamic acid by means of anaerobic alkaline treatment. This was easily accomplished. The product was identified as pteroylglutamic acid by chemical and biological assay and by ultraviolet absorption data.

(1) (a) Cosulich and Smith, THIS JOURNAL, **70**, 1922 (1948); (b) Hultquist, Smith, Seeger, Cosulich and Kuh, *ibid.*, **71**, 619 (1949).

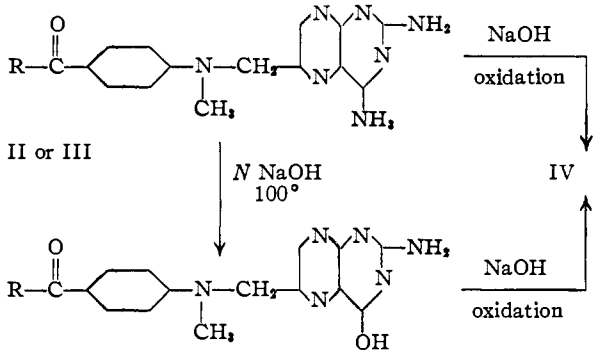
(2) (a) Seeger, Smith and Hultquist, *ibid.*, **69**, 2567 (1947); (b) Smith, Cosulich, Hultquist and Seeger, *Trans. N. Y. Acad. Sciences*, II, **10**, 82 (1948); (c) Minnich and Moore, *Fed. Proc.*, **7**, 276 (1948); (d) Swendseid, *et al.*, *ibid.*, **7**, 299 (1948); (e) Franklin, Stokstad and Jukes, *Proc. Soc. Exptl. Biol. Med.*, **67**, 398 (1948); (f) Little, *et al.*, *J. Lab. Clin. Med.*, **33**, 1144 (1948); (g) Oleson, Hutchings and Subba-Row, *J. Biol. Chem.*, **175**, 359 (1948); (*h*) Farber, *et al.*, *New England J. of Med.*, **238**, 787 (1948).

(3) (a) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948); (b) Hultquist and Dreisbach, U. S. Patent 2,443,165, June 8, 1948.

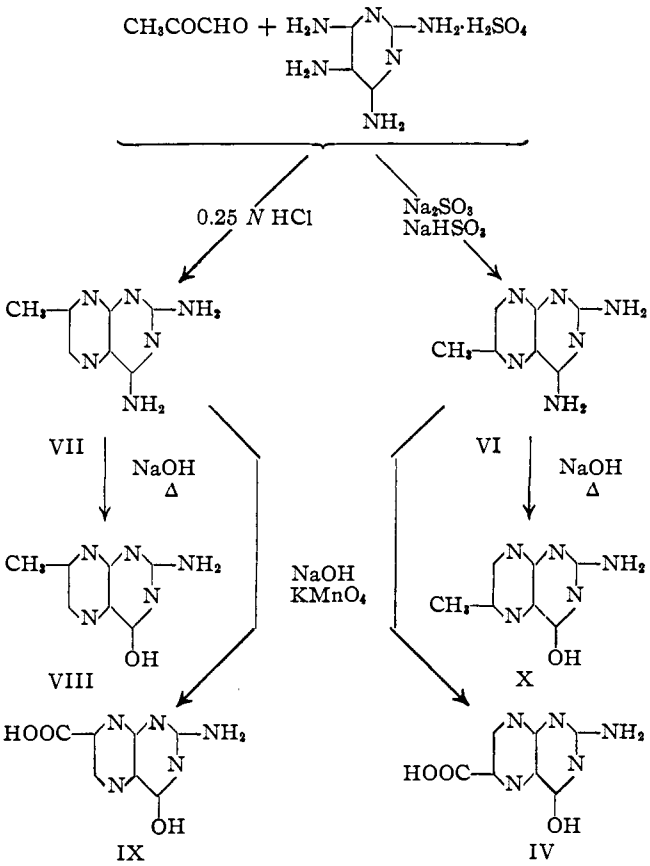
(4) (a) Traube, *Ber.*, **37**, 4545 (1904); (b) Watson and Yates, *J. Chem. Soc.*, 1207 (1932).

(5) Angier, *et al.*, *Science*, **103**, 667 (1946); Stokstad, *et al.*, THIS JOURNAL, **70**, 5 (1948).

(6) Mowat, *et al.*, *ibid.*, **70**, 14 (1948).



Certain 2,4-diaminopteridines exhibiting pteroylglutamic acid antagonism[7] have been described by Mallette, Taylor and Cain.[8a] These included a compound designated as 2,4-diamino-6(or 7)-methylpyrimido[4,5-b]-pyrazine obtained



by the reaction of methylglyoxal with 2,4,5,6-tetraminopyrimidine bisulfite in boiling water. Subsequently this was shown to be the 7-isomer by cleavage to a known methylpyrazine.[8b] In the present investigation both 2,4-diamino-6-methylpteridine (VI) and 2,4-diamino-7-methylpteridine (VII) were synthesized as model compounds. When methylglyoxal was allowed to react with 2,4,5,6-tetraminopyrimidine sulfate in dilute hydrochloric acid solution, the 7-methyl isomer was the chief product. Proof of this was obtained by alkaline anaerobic hydrolysis to 2-amino-4-hydroxy-7-methylpteridine (VIII) and by alkaline permanganate oxidation to 2-amino-4-hydroxypteridine-7-carboxylic acid (IX) both of which are known.[6] A diacetyl derivative was obtained from VII and acetic anhydride.

Semb found that 2-amino-4-hydroxy-6-methylpteridine resulted from the action of methylglyoxal on 2,4,5-triamino-6-hydroxypyrimidine in water solution at *p*H 7 in the presence of sodium sulfite.[9] Similarly, 2,4-diamino-6-methylpteridine was the principal product when 2,4,5,6-tetraaminopyrimidine sulfate was substituted for the triamine. Purification of the initial product was accomplished by extraction with cold 10% acetic acid in which VI is soluble. The small amount of insoluble material consisted largely of the 7-isomer (VII). The purified 6-isomer (VI) was converted to 2-amino-4-hydroxy-6-methylpteridine (X) by heating anaerobically with alkali and to 2-amino-4-hydroxypteridine-6-carboxylic acid (IV) by alkaline permanganate oxidation. Acetylation of VI gave a diacetyl derivative. Absorption data for the compounds VI and VII are given in Table I.

The biological properties of these compounds have been examined by Dr. B. L. Hutchings and Dr. E. L. R. Stokstad, of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. For N-[4-{[(2,4-diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid (I) the inhibition ratio for half-maximum inhibition of the growth of *Streptococcus faecalis* R. is 1.9, 0.7 and 0.4 at concentrations of pteroylglutamic acid of 0.003, 0.005 and 0.01 microgram per 10 ml., respectively. Details of this work will be reported elsewhere.

In Table I are listed the ultraviolet absorption data as well as the relative biological activities of the pure compounds.

Experimental

N-[4-{[(2,4-Diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic Acid (I) ("Aminopterin").—A mixture of 27.4 g. of 2,4,5,6-tetraminopyrimidine sulfate dihydrate,[4a] 24.4 g. of barium chloride dihydrate and 500 ml. of water was heated at 60° for ten minutes, then cooled to 45°. *p*-Aminobenzoyl-l(+)-glutamic acid (13.3 g.) was added, and the *p*H was adjusted to 3 with 5 *N* sodium hydroxide solution. During twenty minutes 21.7 g. of 2,3-dibromopropionaldehyde in acetic acid and an aqueous solution of 12.5 g. of iodine and 25 g. of potassium

(7) (a) Daniel, *et al.*, *J. Biol. Chem.*, **169**, 689 (1947); (b) Daniel and Norris, *ibid.*, **170**, 747 (1947); (c) Daniel, *et al.*, *ibid.*, **173**, 123 (1948).

(8) (a) Mallette, Taylor and Cain, THIS JOURNAL, **69**, 1814 (1947); (b) Cain, Mallette and Taylor, *ibid.*, **70**, 3026 (1948).

(9) J. Semb and co-workers, private communication.

TABLE I

ANTAGONIST ACTIVITY[a] AND ULTRAVIOLET ABSORPTION DATA

Compound	Antagonist activ.	Wavelengths Å: 0.1 *N* HCl Max.	0.1 *N* HCl Min.	0.1 *N* NaOH Max.	0.1 *N* NaOH Min.
N-[4-{[(2,4-Diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid (I)	700.0	244 290 335	234 259 326	260 284 370	239 271 333
4-{N-[2,4-Diamino-6-pteridyl)-methyl]-N-methylamino}-benzoic acid (II)	162.0	240 311	263	259 286 371	272 330
N-[4-{N-[(2,4-Diamino-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid (III)	1530.0	244 307	264	257 302 370	239 273 342
2,4-Diamino-6-methylpteridine (VI)	0.1	242 281 337	265 297	255[b] 369	304 234
2,4-Diamino-7-methylpteridine (VII)	0.1	242 281 332	264 293	254[b] 361	300

[a] An arbitrary value of 100 is assigned for the antagonist activity of N^{10}-methylpteroylglutamic acid (reference 1) for half-maximum inhibition of the growth of *Streptococcus faecalis* R. Values for other compounds are reported in terms of the standard.

[b] The material was first dissolved in the minimum amount of dilute acid, then treated with sodium hydroxide to give a 0.1 *N* sodium hydroxide solution. The solubility under these conditions was sufficient to allow measurements to be made.

iodide were added simultaneously with sodium hydroxide solution to maintain the *p*H at 2.8–3.0. The reaction mixture was maintained at 45° and *p*H 2.8–3.0 for an additional twenty minutes. The *p*H was then adjusted to 4 and the mixture was cooled and filtered. The crude product weighed from 50 to 60 g. and assayed[10] 9 to 12% in different experiments.

I was also prepared by the method of Hultquist and Dreisbach[3b] using tribromoacetone,[4b] the tetraminopyrimidine and *p*-aminobenzoylglutamic acid.

A slurry of 50 g. of this crude material in 2200 ml. of water and 10 ml. of 50% caustic at 80° was treated with an aqueous solution of 10 g. of calcium chloride, clarified and washed with hot water. The filtrate was adjusted to *p*H 10.7 with aqueous zinc chloride, clarified, acidified to *p*H 4 and filtered. This cake was slurried in 2500 ml. of water, made alkaline with caustic at 80° and then adjusted to *p*H 7 while cooling to 20°. After clarification the filtrate was acidified to *p*H 4. The product was filtered and then dissolved in 2000 ml. of water as the magnesium salt, treated with Darco G-60, clarified and reprecipitated at *p*H 4. After one repetition of this treatment, 3.9 g. of product was obtained. The purity at this stage varied from about 70–80%[10] in different experiments.

A sample of I (3 g.) purified in this manner and assaying 79% was slurried with 1.5 g. of magnesium oxide and 1.5 g. of Darco G-60 in 150 ml. of water at 90°, clarified and cooled. The magnesium salt thus obtained was filtered and recrystallized four times from hot water. The product was finally obtained as clusters of yellow needles which were filtered and dried.

Anal. Calcd. for $C_{19}H_{18}O_5N_8Mg\cdot 3H_2O$: C, 44.2; H, 4.7; N, 21.7; Mg, 4.7. Found: C, 44.6; H, 4.85; N, 21.4; Mg, 4.82.

(10) Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).

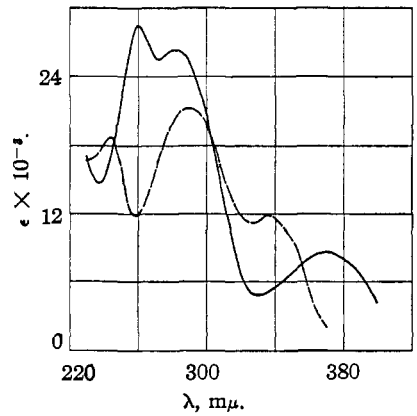


Fig. 1.—Ultraviolet absorption spectra[a] of N-[4-{[(2,4-diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid dihydrate: ——, 0.1 *N* sodium hydroxide; — — —, 0.1 *N* hydrochloric acid.

The free acid was obtained by dissolving the magnesium salt in water, acidifying to *p*H 4, cooling, filtering and drying.

Anal. Calcd. for $C_{19}H_{20}O_5N_8\cdot H_2O$: C, 47.9; H, 5.1; N, 23.5. Found: C, 47.3; H, 5.18; N, 23.4.

Conversion of I to Pteroylglutamic Acid.—A solution of 0.59 g. of I (84.7%) in 20 ml. of *N* sodium hydroxide solution was heated at 100° under nitrogen for six hours. The solution was then cooled, diluted with water, adjusted to *p*H 3 and filtered. The dry product weighed 0.395 g. and assayed 70.1%[10]. Bioassay by Dr. B. L. Hutchings showed 65.5% pteroylglutamic acid. The ultraviolet absorption curve indicated the product to be pteroylglutamic acid with an estimated purity of 70%.

Oxidation of I.—A solution of 0.5 g. of I in 150 ml. of *N* sodium hydroxide was oxidized with excess potassium permanganate at 90–95°. On working up as described for II below, 0.177 g. of 2-amino-4-hydroxypteridine-6-carboxylic acid (IV) was obtained. Oxygen was bubbled through a solution of 0.59 g. of I in 20 ml. of sodium hydroxide at 100° for six hours. The product obtained on acidification (0.235 g.) appeared to be a mixture of IV and X, judging from the ultraviolet absorption spectra.

4-{N-[(2,4-Diamino-6-pteridyl)-methyl]-N-methylamino}-benzoic Acid (II).—A mixture of 26 g. of 2,4,5,6-tetraminopyrimidine sulfate dihydrate[4a] and 24 g. of barium chloride dihydrate in 700 ml. of water was heated at 60° for ten minutes. After cooling to 40°, 15 g. of *p*-methylaminobenzoic acid[1] was added and the *p*H of the mixture adjusted to 3–4 with dilute sodium hydroxide. Then at 40° the simultaneous addition of the following three solutions was carried out over thirty minutes: 21.6 g. of 2,3-dibromopropionaldehyde in 21.6 ml. of acetic acid, 12.5 g. of iodine and 25 g. of potassium iodide in 100 ml. of water, and sodium hydroxide solution to maintain *p*H 3–4. After cooling overnight at 5° the crude material was isolated by filtration with Hyflo-Supercel.

One half of the crude was heated at 60° for forty minutes in one liter of water containing 6 g. of lime. The insoluble material was filtered and washed with 60° water. The filtrate was adjusted to *p*H 3 with dilute hydrochloric acid and allowed to stand in the ice-box overnight. The precipitate, isolated by filtration, was heated at 60° for ten minutes with 750 ml. of water and enough sodium hydroxide to give *p*H 11–12. The *p*H was then adjusted to 7 at 20°. The precipitated material was removed by filtration with Hyflo-Supercel, and the filtrate was adjusted to *p*H 3 with dilute hydrochloric acid. After cooling at 5° overnight the precipitate was filtered off, slurried in 500 ml. of water, and treated with the minimum amount of magnesium oxide to give *p*H 8.8–9.3 at 80° after fifteen minutes of stirring. Then 0.5 g. of Darco G-60 was added

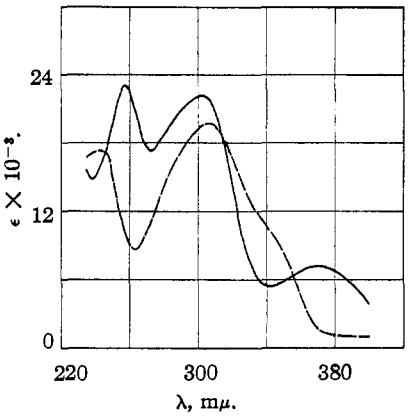


Fig. 2.—Ultraviolet absorption spectra[a] of N-[4-{N-[(2,4-diamino-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid monohydrate: ——, 0.1 *N* sodium hydroxide; — — —, 0.1 *N* hydrochloric acid.

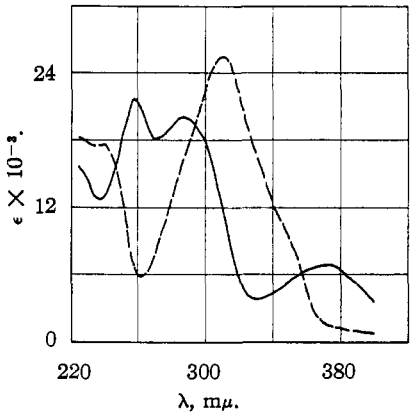


Fig. 3.—Ultraviolet absorption spectra[a] of 4-{N-[(2,4-diamino-6-pteridyl)-methyl]-N-methylamino}-benzoic acid dihydrate: ——, 0.1 *N* sodium hydroxide; — — —, 0.1 *N* hydrochloric acid.

[a] ϵ is the molecular extinction coefficient as defined by $I = I_0 \cdot 10^{-\epsilon cl}$ where c is the concentration in moles/liter and l is the cell length in centimeters. Transmittancy (I/I_0) measurements of 10 mg./l. solutions were made in 1-cm. cell at 5 mμ intervals on a Model DU Beckman 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.

and the heating continued at 80° for fifteen minutes more. The hot filtered solution was adjusted to *p*H 3 with dilute hydrochloric acid and allowed to stand overnight at 5°. The crystalline compound obtained was estimated to be about 88% pure by ultraviolet absorption data. It was purified for analysis by repeating the last step above twice more, and dried at 100° (1 mm.) for six and one-half hours. It had a melting point of 254–255° (dec.).

Anal. Calcd. for $C_{18}H_{16}N_7O_2 \cdot 2H_2O$: C, 49.85; H, 5.27; N, 27.18. Found: C, 50.0; H, 5.55; N, 27.2.

Conversion of II to 4-{N-[(2-Amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoic Acid[1] (V).—A solution of 250 mg. of II in 25 ml. of 1 *N* sodium hydroxide solution was heated at 100° under a rapid stream of nitrogen for six hours. After diluting with water, the solution was adjusted to *p*H 3–4 with 5 *N* hydrochloric acid. The precipitated material was collected by centrifugation, washed and dried, and was identified as V by comparison of its ultraviolet absorption spectrum with that of an authentic sample.[1] The purity was about 85% as estimated from the absorption spectra data.

Alkaline Permanganate Oxidation of II.—To a warmed solution of 500 mg. of II in 166 ml. of *N* sodium hydroxide was added a solution of potassium permanganate until the green color remained after ten minutes. A little sodium hydrosulfite was added to remove this color, and then the manganese dioxide was filtered off. The filtrate was adjusted to *p*H 3–4 and cooled. After centrifugation the wet precipitate was dissolved in the minimum amount of dilute sodium hydroxide. The solution (22.5 ml.) was then made 5 *N* by the addition of solid sodium hydroxide. The crystalline sodium salt was cooled overnight in the ice-box and then collected on vinyon cloth. It was dissolved in water and the solution was clarified with Darco G-60. The pale yellow filtrate was adjusted to *p*H 3–4 with dilute hydrochloric acid. The precipitate when dried weighed 185 mg. and was shown to be 2-amino-4-hydroxypteridine-6-carboxylic acid (IV) by comparison of the ultraviolet absorption spectrum with that of an authentic sample.[6] The purity was estimated to be about 85% from the ultraviolet absorption curve data.

N-[4-{N-[(2,4-Diamino-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic Acid (III) ("A-Methopterin").—A mixture of 27.4 g. of 2,4,5,6-tetraaminopyrimidine sulfate dihydrate[4a] and 24.4 g. of barium chloride dihydrate in 700 ml. of water was heated to 60° for ten minutes. After cooling to 40°, 32.4 g. of disodium *p*-methylaminobenzoylglutamic acid[1] was added. Dilute sodium hydroxide was then added to adjust to *p*H 3–3.5. Then at 40° were added 21.6 g. of 2,3-dibromopropionaldehyde in 21.6 ml. of acetic acid, 12.5 g. of iodine and 25 g. of potassium iodide in 100 ml. of water, and sodium hydroxide solution as necessary to maintain *p*H 3–3.5. The resulting mixture was cooled overnight in the ice-box, and then filtered.

One-half of the crude was heated at 60° for forty minutes with 2 l. of water and 9 g. of lime. The filtered solid was washed with 750 ml. of water at 60°, and the filtrate was adjusted to *p*H 3.5–4.0 with dilute hydrochloric acid and allowed to stand overnight at 5°. The precipitate was filtered and slurried in 1500 ml. of water with enough sodium hydroxide to give *p*H 11–12 at 60°. After adjustment to *p*H 7 at 20° and clarification, the filtrate was brought to *p*H 3–4 with dilute hydrochloric acid and cooled at 5° overnight. The precipitate was slurried in 1 l. of water and heated for fifteen minutes at 60° with the minimum amount of lime to give *p*H 9.0–9.4. Darco G-60 (1 g.) was then added and heating continued for fifteen minutes longer. The filtered solution was reheated to 60° and dilute hydrochloric acid added to *p*H 3–4. After cooling at 5° overnight, the yellow-orange microcrystalline material was collected and dried. It was 87% pure by ultraviolet absorption curve data. This material was purified for analysis by recrystallization from hot water containing a little hydrochloric acid,[11] and dried at 100° (3 mm.) for four hours. When inserted into the bath preheated to 160° it melted with decomposition at 185–204°.

Anal. Calcd. for $C_{20}H_{22}N_8O_6 \cdot H_2O$: C, 50.75; H, 5.07; N, 23.7. Found: C, 50.7; H, 5.23; N, 23.5.

Conversion of III to N-[4-{N-[(2-Amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic Acid (XI).—A solution of 250 mg. of III in 25 ml. of *N* sodium hydroxide solution was heated at 100° under a rapid stream of nitrogen for four and one-half hours. The diluted solution was adjusted to *p*H 3–4 with 5 *N* hydrochloric acid. The precipitated material, isolated by filtering, washing and drying, was identified as XI, 85% pure by comparison of its ultraviolet absorption spectrum with that of an authentic sample.[1]

2,4-Diamino-7-methylpteridine (VII).—A solution of 27 g. of 2,4,5,6-tetraaminopyrimidine sulfate dihydrate[4a] in 2000 ml. of 0.25 *N* hydrochloric acid was warmed to 40° and 25 ml. of commercial 30% methylglyoxal solution was added all at once. A deep golden yellow color developed

(11) Hutchings, *et al.*, THIS JOURNAL, **70**, 1 (1948).

immediately. After thirty minutes 130 g. of 50% sodium hydroxide solution was added and the solution was cooled in the ice-bath. The yellow product was collected on the filter, washed well with water and then with acetone, and dried at 50°. The yield was 11 g. (62.5%). For analysis a small sample was dried for six hours at 100° (1 mm.).

Anal. Calcd. for $C_7H_8N_6$: C, 47.72; H, 4.57; N, 47.71. Found: C, 47.6; H, 4.8; N, 47.8.

Oxidation of VII.—One gram of VII in 250 ml. of *N* sodium hydroxide solution at 80–90° was treated with 2% potassium permanganate solution until the green color persisted. This required about five minutes. A little sodium bisulfite was added to destroy the green color, and manganese dioxide was removed by filtration. The yellow solution was acidified with dilute hydrochloric acid and the finely divided precipitate collected and redissolved in 800 ml. of very dilute sodium hydroxide solution. It was treated with a little Darco G-60 and filtered. The filtrate was heated to 90°, dilute hydrochloric acid was added to adjust the solution to *p*H 3.5–4.0, and after cooling overnight in the ice-box the precipitate was collected, washed with water and with acetone, and dried at 50°. The yield was 0.71 g. (61%). For analysis a small sample was dried three hours at 100° (1 mm.).

Anal. Calcd. for $C_7H_5N_5O_3$: C, 40.58; H, 2.83; N, 33.81. Found: C, 40.8; H, 3.07; N, 34.0.

The oxidation product was identified as 2-amino-4-hydroxypteridine-7-carboxylic acid (IX) by comparison with a known sample.[6]

Conversion of VII to 2-Amino-4-hydroxy-7-methylpteridine) (VIII).—A sample of VII (0.5 g.) was dissolved in 40 ml. of water with a minimum amount of hydrochloric acid, diluted to 80 ml. with water and treated with 20 ml. of 5 *N* sodium hydroxide solution. A yellow solution resulted from which a small amount of yellow material began to crystallize slowly. The temperature was adjusted to 100° and a fairly rapid stream of oxygen was bubbled through for four hours. The resulting yellow solution was acidified and the precipitate was collected in the centrifuge and washed with water. It was redissolved in very dilute sodium hydroxide solution at *p*H 11–12, treated with Darco G-60, filtered, and the filtrate was acidified. The product was collected in the centrifuge, washed with water and dried. The yield was 0.35 g. This product was identified as 2-amino-4-hydroxy-7-methylpteridine (VIII) by comparison with an authentic sample.[6] This conversion was also carried out similarly in *N* sodium hydroxide and in *N* sulfuric acid under anaerobic conditions.

2,4-Diacetamido-7-methylpteridine.—A sample of VII (2 g.) was slurried in 50 ml. of acetic anhydride and the mixture heated to boiling. A red solution resulted. It was cooled and the sandy pink precipitate was filtered off, washed with ethanol and with ether, and dried. The yield was 1.45 g. This was recrystallized from 200 ml. of boiling ethanol with decolorization by Darco G-60. The diacetyl compound precipitated almost at once as fine white powder. It was collected on the filter, washed with alcohol, and dried at 50°. The yield was 0.79 g., m. p. 236–237°.

Anal. Calcd. for $C_{11}H_{12}N_6O_2$: C, 50.76; H, 4.64; N, 32.3. Found: C, 50.6; H, 4.65; N, 32.0.

2,4-Diamino-6-methylpteridine (VI).—The method used is essentially that of Semb, *et al.*[9] A slurry of 26 g. of 2,4,5,6-tetraaminopyrimidine sulfate dihydrate and 260 g. of sodium sulfite in 900 ml. of water was heated to 60°; most of the solids dissolved. It was cooled to 30° and 100 ml. of an aqueous solution of 22 ml. of methylglyoxal (30%) containing 5 g. of sodium bisulfite added at once. After forty minutes at room temperature, the yellow precipitate was filtered and washed three times by reslurrying in 500 ml. of water. The washed and dried material weighed 12.4 g., representing a crude yield of 70.5%.

A 5-g. sample of the crude was purified by stirring one-half hour in 250 ml. of 10% acetic acid. The insoluble material when washed and dried, weighed 0.475 g. and was found to be largely 2,4-diamino-7-methylpteridine (VII). The yellow acetic acid solution was clarified with Darco G-60 and adjusted to *p*H 6.4 with ammonium hydroxide. After cooling overnight in the ice-box, the precipitate was centrifuged, washed with water, then acetone, and dried at 100° at 2 mm. for three hours. The material weighed 2.2 g.

Anal. Calcd. for $C_7H_8N_6$: C, 47.72; H, 4.57; N, 47.71. Found: C, 47.5; H 4.81; N, 47.4.

Oxidation of VI.—A slurry of 500 mg. of VI in 166 ml. of *N* sodium hydroxide solution was warmed on the steam-bath several hours to give partial solution. Then to the mixture at the boiling point was added potassium permanganate solution slowly until the change of color from purple to dark green was very slow. A little sodium hydrosulfite was then added to remove the dark green color and the manganese dioxide was filtered off. The filtrate was adjusted to *p*H 3 and cooled overnight. The yellow precipitate was centrifuged, washed with water, and the wet cake was treated with just enough 5 *N* sodium hydroxide solution to obtain complete solution. Then the solution was made 5 *N* by adding solid sodium hydroxide. After cooling overnight in the ice-box, the crystalline disodium salt was filtered and washed with a little alcohol. It was dissolved in water and the solution clarified with Darco G-60, and acidified with acetic acid. The precipitate, when washed and dried, weighed 190 mg. and was shown to be 2-amino-4-hydroxypteridine-6-carboxylic acid (IV) by comparison of the ultraviolet absorption curve with that of an authentic sample.[6]

Conversion of VI to 2-Amino-4-hydroxy-6-methylpteridine (X).—A mixture of 500 mg. of VI and 25 ml. of *N* sodium hydroxide solution was heated at 100° under nitrogen for six hours, at the end of which time solution was completed. After clarifying, adjusting to *p*H 4, and cooling overnight, the precipitate was centrifuged out and dissolved by adding the minimum amount of dilute sodium hydroxide. The solution was clarified and made 5 *N* by adding solid sodium hydroxide. The crystalline sodium salt, which separated out on cooling overnight, was filtered off and dissolved in water. The solution was clarified with Darco G-60 and acidified with acetic acid. The yellow precipitate, when washed and dried, weighed 400 mg. and was shown to be 2-amino-4-hydroxy-6-methylpteridine (X) by comparison of the ultraviolet absorption curve with that of an authentic sample.[6]

2,4-Diacetamido-6-methylpteridine.—A mixture of 1 g. of VI in 25 ml. of acetic anhydride was boiled until a dark pink solution resulted. On cooling several hours a pink crystalline precipitate appeared which was filtered, washed with a small amount of acetic anhydride and then with cold ethanol. The dried crude material weighed 0.85 g. and was recrystallized twice from ethanol with Darco G-60 clarifications to give 0.165 g. of white crystalline material with a melting point of 234.5–236.5°. It shows a marked depression in a mixture melting point with 2,4-diacetamido-7-methylpteridine.

Anal. Calcd. for $C_{11}H_{12}N_6O_2$: C, 50.76; H, 4.64; N, 32.3. Found: C, 50.6; H, 4.64; N, 32.3.

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Summary

N-[4-{[(2,4-Diamino-6-pteridyl)-methyl]-amino}-benzoyl]-glutamic acid (I), 4-{N-[(2,4-diamino-6-pteridyl)-methyl]-N-methylamino}-benzoic acid (II) and N-[4-{N-[(2,4-diamino-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid (III) have been synthesized in pure form. They are powerful antagonists of the growth-promoting action of pteroylglutamic acid for *S. faecalis* R.

The structures were proved by degradation to known compounds. In these reactions it was observed that the 4-amino group in 2,4-diaminopteridines was readily converted to hydroxyl.

Anaerobic alkaline treatment of I resulted in the formation of pteroylglutamic acid, thus constituting a new synthesis of the latter compound.

The previously reported reaction of methylglyoxal with 2,4,5,6-tetraminopyrimidine was investigated, and it was found that in 0.25 *N* hydrochloric acid 2,4-diamino-7-methylpterin was the chief product while in sodium sulfite solution at *p*H 7 the 6-methyl isomer predominated.

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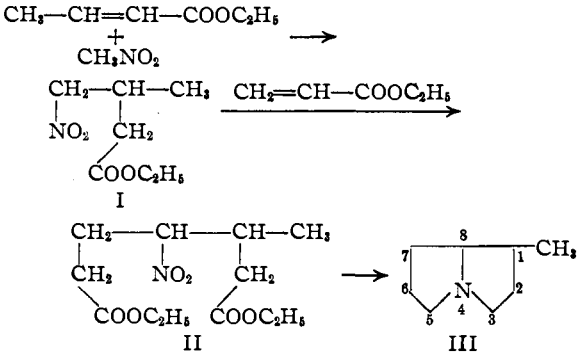
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Synthesis of Pyrrolizidines. III. Condensation of Nitromethane with Ethyl Crotonate and Subsequent Formation of 1-Methylpyrrolizidine (Heliotridane, Pseudoheliotridane)[1]

BY NELSON J. LEONARD AND DONALD L. FELLEY

The compound 1-methylpyrrolizidine (III) occupies an important position in alkaloid chemistry because it has been obtained, in the optically active form called *l*-heliotridane, as a degradation product of a large number of alkaloids found in the *Senecio, Heliotropium, Crotalaria, Erechtites,* and *Trichodesma* genera. Men'shikov[2] first showed that *l*-heliotridane probably possessed structure III, and Adams and Rogers[3] established this structure unequivocally. More recently, Men'shikov and Borodina[4] have shown that *l*-pseudoheliotridane, from the alkaloids of *Trachelanthus korolkovi*, is probably the diastereoisomer of *l*-heliotridane. The first synthesis of 1-methylpyrrolizidine (III) was effected by Men'shikov[5] in amount sufficient only to characterize the product as its picrate. Prelog and Zalan[6] have since synthesized this compound by a five-step procedure, starting with γ-ethoxypropyl bromide and γ-phenoxy-α-methylbutyronitrile, in a 22% over-all yield. In both syntheses, apparently only one of the two expected diastereoisomeric racemates, "*dl*-heliotridane," was obtained.

The reductive cyclization of diethyl β-methyl-γ-nitropimelate (II), by the method developed in this Laboratory for the synthesis of pyrrolizidines,[1] suggested itself as a more convenient and efficient method for the synthesis of 1-methylpyrrolizidine. This method has been found to be practical, and 1-methylpyrrolizidine has been synthesized, starting with ethyl crotonate and nitromethane, in an over-all yield of 26%. It was found that the addition of nitromethane to ethyl crotonate to yield ethyl β-methyl-γ-nitrobutyrate (I) could not be effected using either the method of Bruson[7] for acrylic esters or the method of Kohler and Engelbrecht[8] for certain more highly activated α,β-unsaturated esters. With diethylamine as a catalyst,[9] in the method used by Kloetzel[10] for the condensation of nitroparaffins with α,β-unsaturated ketones, a yield of 15% of I was obtained. It seemed possible that the use of diisopropylamine as a catalyst might result in a better yield of the product since this amine is of commensurate strength with diethylamine[11] but would probably not be as active in the competing reaction of addition of the amine to the ethyl crotonate.[12] Using diisopropylamine in the condensation of nitromethane with ethyl crotonate, a 25% yield of I was obtained. Other amines were studied as catalysts in this condensation but the best yield (55%) was obtained by the use of benzyltrimethylammonium butoxide in butanol at 75–80°.



The condensation of ethyl β-methyl-γ-nitrobutyrate (I) with ethyl acrylate to give diethyl β-

(1) For the first two papers in this series, see (a) Leonard, Hruda and Long, THIS JOURNAL, **69**, 690 (1947); (b) Leonard and Beck, *ibid.*, **70**, 2504 (1948).

(2) (a) Men'shikov, *Ber.*, **66**, 875 (1933); (b) **68**, 1051 (1935); (c) **69**, 1799 (1936); (d) **69**, 1802 (1936); (e) *J. Gen. Chem.* (U. S. S. R.), **7**, 1632 (1937).

(3) Adams and Rogers, THIS JOURNAL, **63**, 228 (1941).

(4) (a) Men'shikov and Borodina, *J. Gen. Chem. (U. S. S. R.)*, **15**, 225 (1945); (b) **16**, 1311 (1946).

(5) Men'shikov, *Bull. acad. sci. U. S. S. R., Classe sci. math. nat., Ser. chim.*, **5**, 1035 (1937).

(6) Prelog and Zalan, *Helv. Chim. Acta*, **27**, 531 (1944).

(7) Bruson, U. S. Patent 2,342,119, Feb. 22, 1944.

(8) Kohler and Engelbrecht, THIS JOURNAL, **41**, 764 (1919).

(9) Worrall and Bradway, *ibid.*, **58**, 1607 (1936).

(10) Kloetzel, *ibid.*, **69**, 2271 (1947).

(11) Hall and Sprinkle, *ibid.*, **54**, 3469 (1932).

(12) Hromatka, *Ber.*, **75**, 131 (1942), found that diisopropylamine does not react with ethyl acrylate, while Flürscheim, *J. prakt. Chem.*, [2] **68**, 348 (1903), found that diethylamine reacts with ethyl acrylate at reflux temperature to give a quantitative yield of ethyl β-diethylaminopropionate after one hour.