with carbon and cooled with ice. By direct crystallization 8 g. is obtained which recrystallized in absolute alcohol; m. p. 153–155° (according to Reichstein[2] 157–158°). Ammoniacal silver solution is reduced, there is no precipitate with digitonin, and it contains chlorine. *Anal.* Found: C, 70.6; H, 8.6. Calcd. as $C_{23}H_{33}O_3Cl$: C, 70.3; H, 8.5. The acid hydrolysis of this product followed by oxidation of the hydroxyl group and replacement of the chlorine by an acetoxy group according to the methods of Reichstein gave desoxycorticosterone acetate.

Benzoate of Δ⁵-Pregnenol-3-one-20.—To a solution of 10 g. of pregnenolone in 150 cc. of anhydrous pyridine and cooled with ice, 10 g. of benzoyl chloride is added and the mixture is left at room temperature for twenty-four hours. After isolation and separation of the excess benzoic acid, the moist product is crystallized in a large volume (about one liter) of ethyl acetate or of acetone–water. The yield is 11 g., m. p. 192–193°. It forms pretty rectangular strips, $[\alpha]^{20}D$ +56° (chloroform). *Anal.* Found: C, 80.2; H, 8.9. Calcd. as $C_{28}H_{36}O_3$: C, 80.0; H, 8.6. It has the same m. p. as free pregnenolone (191–193°) but the mixture drops to 165–175°. Furthermore from the differences in crystalline form and solubility, pregnenolone benzoate is distinguished clearly from pregnenolone in that it does not precipitate with digitonin. Pregnenolone benzoate is only slightly soluble in alcohol, ether and petroleum ether. It is moderately soluble in acetone and ethyl acetate and very soluble in benzene, carbon tetrachloride and in chloroform.

3-Benzoate-21-acetate of Δ⁵-Pregnendiol-3,21-one-20.—To a solution of 6 g. of pregnenolone benzoate in 300 cc. of glacial acetic acid is added 30 g. of lead tetraacetate and 50 cc. of acetic anhydride. The mixture is heated in water-bath, absolutely water free, for twenty-four hours. It is poured over one and one-half liters of water containing a small amount of ice and extracted with isopropyl ether. The ether solution is washed with dilute sodium hydroxide solution, then with water, dried and the ether evaporated. Reacting differently from the original benzoate, the new substance is much more soluble in ether. The residue after evaporation of the solvent is recrystallized several times in alcohol–water. The yield is 1.5 g., m. p. 175–176°, $[\alpha]^{20}D$ +161° (chloroform). *Anal.* Found: C, 75.7; H, 8.3. Calcd. as $C_{30}H_{38}O_5$: C, 75.3; H, 8.0.

Acknowledgment.—The microanalyses were carried out in the Instituto Politécnico Nacional of Mexico by Mrs. Leontina Norymberska to whom I am pleased to express my gratitude.

Summary

Partial hydrolysis of the diacetate of Δ⁵-pregnendiol-3,21-one-20 removes the 21-acetoxy group. Treatment of this product with thionyl chloride gave 21-chloropregnenol-3-one-20 acetate, an intermediate in the preparation of desoxycorticosterone acetate by known methods. Several different esters on C_{21} of the 3-monoacetate of Δ⁵-pregnendiol-3,21-one-20 have been prepared. The partial saponification of all these products invariably regenerates the 3-monoacetate. The preparation of pregnenolone benzoate and its reaction with lead tetraacetate are described.

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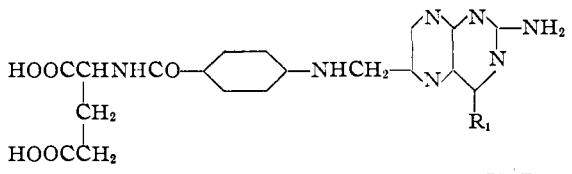
[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Analogs of Pteroylglutamic Acid. V. 4-Alkylamino Derivatives

BY BARBARA ROTH, JAMES M. SMITH, JR., AND MARTIN E. HULTQUIST

The synthesis of N-[4-(2,4-diamino-6-pteridylmethyl)-aminobenzoyl]-glutamic acid (I) ("Aminopterin," "4-aminopteroylglutamic acid") has been described in earlier communications from this Laboratory.[1] This substance has proved to be a powerful antagonist for pteroylglutamic acid. The use of pteroylglutamic acid antagonists in the treatment of blood dyscrasias such as leukemia was proposed by Franklin, *et al.*[2] Meyer[3] reported the treatment of leukemic patients with the "antifolic compounds" pteroylaspartic acid[4a] and N^{10}-methylpteroic acid,[4b] and observed short-lived remissions in a few cases. These two substances were also employed by Farber[5] with similar observations. Farber[5] and subsequently others[6] have obtained temporary remissions in acute leukemia by treatment with 4-aminopteroylglutamic acid (I). Toxic effects are a serious disadvantage in continued use of the drug. In more recent papers Farber[7] and Dameshek[8] have described the use of 4-amino-N^{10}-methylpteroylglutamic acid,[1b] 4-aminopteroylaspartic acid,[9] and 4-amino-9-methylpteroylglutamic acid,[10] with similar observations.

Since I differs from pteroylglutamic acid only in having an amino group in the 4-position in place of the hydroxyl, modification of the amino group



Pteroylglutamic acid, R_1 = OH; I, R_1 = NH_2; II, R_1 = —$NHCH_3$; III, R_1 = —$N(CH_3)_2$; IV, R_1 = —NC_5H_{10}.

(1) (a) Seeger, Smith and Hultquist, THIS JOURNAL, **69**, 2567 (1947); (b) Seeger, Cosulich, Smith and Hultquist, *ibid.*, **71**, 1753 (1949).

(2) Franklin, Stokstad, Belt and Jukes, *J. Biol. Chem.*, **169**, 427 (1947).

(3) Meyer, *Trans. N. Y. Academy of Science*, II, **10**, 99 (1948).

(4) (a) Hutchings, *et al.*, *J. Biol. Chem.*, **170**, 323 (1947); (b) Cosulich and Smith, THIS JOURNAL, **70**, 1922 (1948).

(5) Farber, *et al.*, *New England Journal of Medicine*, **238**, 787–793 (1948).

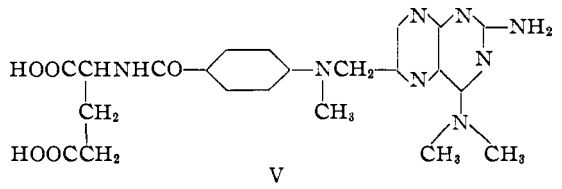
(6) (a) Editorial, *Blood*, **3**, 1057 (1948); (b) Jacobson, Levin, and Holt, *J. Lab. Clin. Med.*, **33**, 1641 (1948); (c) Pierce and Alt, *ibid.*, **33**, 1642 (1948); (d) Berman, *et al.*, *ibid.*, **33**, 1643 (1948); (e) Taylor, *et al.*, *ibid.*, **33**, 1645 (1948); (f) Bethell, Meyers and Neligh, *ibid.*, **33**, 1477 (1948).

(7) Farber, *Blood*, **4**, 160 (1949).

(8) Dameshek, *ibid.*, **4**, 168 (1949).

(9) Hutchings, *et al.*, *J. Biol. Chem.*, **180**, 857 (1949).

(10) Seeger, Hultquist and Smith, unpublished.



V

was undertaken, in an effort to prepare less toxic derivatives. Analogs have been synthesized with methylamino (II), dimethylamino (III), and piperidyl (IV) groups in the 4-position on the pteridine ring. In addition, 4-dimethylamino-N^{10}-methylpteroylglutamic acid (V) has been prepared.

2,4-Diamino-6-chloropyrimidine (VI)[11] was prepared by the chlorination of 2,4-diamino-6-hydroxypyrimidine[12] with phosphorus oxychloride. The 6-alkylaminopyrimidines were then obtained by reaction of VI with methylamine, dimethylamine, and piperidine, respectively. The preparation of the 6-dimethylaminopyrimidine proceeded very smoothly in alcohol at 170°. When the same conditions were used for the 6-methylamino compound, a product was obtained which by analysis was a bismethylaminopyrimidine. At lower temperatures the desired product was obtained in low yields. No difficulty was encountered in the reaction of VI with piperidine at atmospheric pressure.

The 2,4-diamino-6-alkylaminopyrimidines were readily nitrosated in the 5-position, and reduced to yield 2,4,5-triamino-6-alkylaminopyrimidines which were conveniently isolated as sulfates.

Reaction of 2,4,5-triamino-6-dimethylaminopyrimidine (VII) and the corresponding 6-(1-piperidyl) compound with diacetyl yielded the 6,7-dimethylpteridines. 2-Amino-4-dimethylamino-6,7-diphenylpteridine was prepared similarly from benzil and VII.

The 4-alkylamino analogs of pteroylglutamic acid were synthesized by the reaction of Waller, *et al.*,[13] using 2,3-dibromopropionaldehyde, *p*-aminobenzoylglutamic acid,[14] and the appropriate 2,4,5-triamino-6-alkylaminopyrimidine. Purification of III and IV was accomplished by modifications of procedures previously reported. In contrast to the majority of the derivatives of pteroylglutamic acid, N-[4-(2-amino-4-dimethylamino-6-pteridylmethyl)-aminobenzoyl]-glutamic acid (III) has a fairly sharp melting point, and a high solubility in certain organic solvents such as dimethylformamide. Oxidation of III with alkaline permanganate yielded 2-amino-4-hydroxypteridine-6-carboxylic acid,[15] as did oxidation of the 4-(1-piperidyl)-analog (IV). These reactions located the side chain in the 6-position on the pteridine ring.

The synthesis of pteroylglutamic acid by anaerobic alkaline treatment of the 4-amino analog was reported by Seeger, Cosulich, Smith and Hultquist.[1b] In the case of the 4-alkylamino derivatives described in the present paper, a similar conversion was accomplished. 4-Dimethylaminopteroylglutamic acid (III) and the 4-(1-piperidyl) analog (IV), when heated in sodium hydroxide solution under anaerobic conditions, yielded pteroylglutamic acid.

A methylated derivative of III was prepared by a variation of the Waller reaction.[4b] 2,4,5-Triamino-6-dimethylaminopyrimidine, 2,3-dibromopropionaldehyde and *p*-methylaminobenzoylglutamic acid[4b] gave 4-dimethylamino-N^{10}-methylpteroylglutamic acid (V), the 4-dimethylamino analog of 4-amino-N^{10}-methylpteroylglutamic acid ("A-Methopterin").

The biological activity of these compounds has 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, and the details of this work will be reported elsewhere. It can be noted here that the 4-alkylamino compounds have a much lower antagonist activity for *S. faecalis* R, than does 4-aminopteroylglutamic acid.

It is extremely interesting that the biological activity of pteroylglutamic acid can be changed so dramatically by replacing the 4-hydroxyl group with amino, and that substitution on the 4-amino group results in a marked reduction in the antagonist activity.

Experimental

2,4-Diamino-6-chloropyrimidine.[11]—To 400 ml. of phosphorus oxychloride at 80–90° was slowly added 100 g. of 2,4-diamino-6-hydroxypyrimidine. The mixture was then heated at refluxing temperature until all was in solution, which required approximately two hours. Part of the phosphorus oxychloride was then distilled off under vacuum, and the residual syrup poured on ice. The solution was then neutralized with sodium carbonate to about *p*H 8, giving a total volume of approximately 1800 ml. On cooling, a heavy precipitate was formed, which was filtered off and dried. This precipitate contained a considerable quantity of inorganic salts in addition to the 2,4-diamino-6-chloropyrimidine; the latter was extracted from the mixture with three 1 l. portions of hot acetone. A portion of the product crystallized on cooling the acetone solution, and the remainder was recovered by evaporation to dryness. The yield was 59 g. (66%) of a product with a melting point of 197–200°. This was used without further purification in subsequent reactions.

2,4-Diamino-6-dimethylaminopyrimidine.—A mixture of 100 g. of 2,4-diamino-6-chloropyrimidine and 200 g. of dimethylamine in 1 l. of absolute alcohol was heated in a steel autoclave at 170° for three hours. On cooling the resultant solution, a heavy crystalline precipitate formed, which was filtered off and dried. This weighed 102 g. and upon recrystallization from alcohol melted between 153–173°. This material was very soluble in water, contained halogen, and gave off dimethylamine when an excess of sodium hydroxide was added. Indications were that it was a double compound of 2,4-diamino-6-dimethylaminopyrimidine with dimethylamine hydrochloride.

Anal. Calcd. for $C_6H_{11}N_5 \cdot (CH_3)_2NH \cdot HCl$: C, 40.9;

(11) Gabriel, *Ber.*, **34**, 3363 (1901); Büttner, *ibid.*, **36**, 2232 (1903); Hull, Lovell, Openshaw and Todd, *J. Chem. Soc.*, 41–52 (1947).

(12) Traube, German Patent 134,984; *Friedlander*, **6**, 1191.

(13) Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948); *Science*, **103**, 667 (1946).

(14) Van der Scheer and Landsteiner, *J. Immunology*, **29**, 373 (1935).

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

H, 8.16; N, 35.8; Cl, 15.1. Found: C, 39.1, 39.3; H, 8.11, 8.06; N, 36.0; Cl, 15.3.

The substance was dissolved in water, filtered from a small amount of insoluble material, and a large excess of 50% sodium hydroxide solution added, resulting in the formation of a white precipitate of 2,4-diamino-6-dimethylaminopyrimidine. It was filtered off after cooling to 15°. After two recrystallizations from alcohol, it melted at 193–194.5°.

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.0; H, 7.24; N, 45.7. Found: C, 47.3; H, 7.25; N, 46.0.

2,4-Diamino-5-nitroso-6-dimethylaminopyrimidine.—Ninety-five grams of 2,4-diamino-6-dimethylaminopyrimidine–dimethylamine hydrochloride complex was converted to the free base as above. The wet cake was dissolved in 500 ml. of water, adjusted to *p*H 4 with 5 *N* sulfuric acid and sodium hydroxide, and 20 ml. of glacial acetic acid was then added. The mixture was heated to 80°, and a solution of 30 g. of sodium nitrite in 75 ml. of water was added dropwise, the addition being stopped when a permanent spot on starch–potassium iodide paper was reached. A shiny dark red precipitate formed, which was filtered off after cooling to room temperature, washed with water, and air dried. This weighed 67.2 g., representing a 91% yield of product melting with decomposition at 258–259°.

Anal. Calcd. for $C_6H_{10}N_6O$: C, 39.6; H, 5.53; N, 46.2. Found: C, 39.6; H, 5.61; N, 46.3.

2,4,5-Triamino-6-dimethylaminopyrimidine Sulfate.—To a suspension of 67 g. of 2,4-diamino-5-nitroso-6-dimethylaminopyrimidine in 500 ml. of water was added just enough 5 *N* hydrochloric acid to dissolve the solid. The solution was heated to 50°, and 150 g. of sodium dithionite was slowly added. The maximum temperature reached from the exothermic reaction was 58°. The resultant yellow solution was acidified to *p*H 2 with 1 to 1 (by volume) sulfuric acid. The solution was then allowed to cool to 5° for three hours, resulting in the formation of a heavy white precipitate. This was filtered off and washed with ice water, after which it was dried at 45°. The product, which darkened somewhat on drying, weighed 95.7 g., corresponding to a 98% crude yield. A sample was purified for analysis by reprecipitation from an alkaline solution, which yielded a white crystalline product. It was somewhat unstable, and freshly prepared material was used in the experiments described below.

Anal. Calcd. for $C_6H_{12}N_6 \cdot H_2SO_4$: C, 27.1; H, 5.30; S, 12.0. Found: C, 26.8; H, 5.05; S, 11.6.

2-Amino-4-dimethylamino-6,7-diphenylpteridine.—Two grams of 2,4,5-triamino-6-dimethylaminopyrimidine sulfate was slurried in 20 ml. of water with a trace of sodium dithionite and an equivalent amount of barium chloride, warmed to 50° for ten minutes, and filtered from barium sulfate. A solution of 1.53 g. of benzil in 25 ml. of hot alcohol was added to the filtrate. The mixture was heated to refluxing for four hours, giving a clear orange solution. It was then cooled and neutralized with ammonium hydroxide. A yellow precipitate was obtained which was filtered off, washed with water, and then with alcohol to remove unreacted benzil. The dry weight was 1.8 g. The product was recrystallized from dimethylformamide-water mixtures to give yellow crystals which gradually sintered at about 315° and melted at 322–325°.

Anal. Calcd. for $C_{20}H_{18}N_6$: C, 70.1; H, 5.30; N, 24.6. Found: C, 70.0; H, 5.12; N, 24.9. The substance showed ultraviolet absorption maxima at 279 and 375 mμ in 0.1 *N* hydrochloric acid.

2-Amino-4-dimethylamino-6,7-dimethylpteridine.—One gram of 2,4,5-triamino-6-dimethylaminopyrimidine sulfate plus a trace of sodium dithionite was slurried in 15 ml. of water and ammonium hydroxide added until the mixture was faintly alkaline. The solution was heated to 80°, and 0.32 g. of diacetyl dissolved in 3 ml. of water was added dropwise, causing an orange color to develop, followed by the formation of a precipitate. The mixture was heated for ten minutes after the addition was complete, cooled and filtered. The product was recrystallized from dimethylformamide twice, and then melted with decomposition at 284–285°.

Anal. Calcd. for $C_{10}H_{14}N_6$: C, 55.0; H, 6.46; N, 38.5. Found: C, 55.2; H, 6.63; N, 38.4.

The substance showed ultraviolet absorption maxima at 267 and 371 mμ in 0.1 *N* sodium hydroxide; in 0.1 *N* hydrochloric acid it showed a maximum at 340 mμ with plateaus at 250 and 300 mμ.

4-Dimethylaminopteroylglutamic Acid (III).—A mixture of 180 g. of 2,4,5-triamino-6-dimethylaminopyrimidine sulfate, 90 g. of *p*-aminobenzoylglutamic acid, and 3 l. of water was heated to 45° and adjusted to *p*H 3. To this was added dropwise (and simultaneously), over a twenty-minute period, a solution of 33.7 g. of sodium dichromate in 400 ml. of water and a solution of 146 g. of α,β-dibromopropionaldehyde in 139 g. of glacial acetic acid. The *p*H of the mixture was maintained at 3 throughout by the addition of 5 *N* sodium hydroxide as needed. The mixture was heated at 45° at *p*H 3 for twenty minutes longer, adjusted to *p*H 3.8, and cooled to 6°. The precipitate was filtered after one and one-half hours and washed with water and acetone. Approximately 180 g. of a golden brown product was obtained which by chemical assay[16] contained 37 g. of 4-dimethylaminopteroylglutamic acid. Purification was accomplished by the following procedure. The crude product was added to 12 l. of water at 80°, and 325 ml. of 5 *N* sodium hydroxide added to effect solution. This was stirred for ten minutes, and 45 g. of calcium chloride in 110 ml. of water was added. The precipitate was filtered with the help of Hyflo–Supercel and washed well with hot water. The filtrate at 60° was adjusted to *p*H 10.8–11.0 with 10% zinc chloride solution. Insolubles were removed by filtration with Hyflo–Supercel and the filtrate was neutralized to *p*H 4 with hydrochloric acid. The mixture was cooled to 3° for an hour and then filtered with Hyflo–Supercel. The cake was slurried in 6700 ml. of water at room temperature and 68 g. of lime added. It was stirred at room temperature for ten minutes and then heated to 60°, filtered, and washed with hot water. To the filtrate at 60° was added 10% zinc chloride solution, to *p*H 10.8–11.0, and the mixture then filtered with Hyflo–Supercel. The filtrate was heated to 80° and neutralized to *p*H 3.6 followed by filtration of the hot solution from some tarry material. The filtrate was cooled to 6° and filtered with the aid of Hyflo–Supercel after two hours. The precipitate was slurried in 3400 ml. of water and warmed slowly to 60° while adding magnesium oxide slowly to about *p*H 8. After five minutes, 5 g. of Darco was added. The mixture was stirred at 60° for fifteen minutes longer, and then filtered. The filtrate was neutralized to *p*H 3.8 at 80° and filtered hot. The solution was cooled to 6°, filtered, and the solid washed with water and acetone. After drying at 45°, the yellow product weighed 9.2 g. and had a chemical assay of 69.6% as 4-dimethylaminopteroylglutamic acid. A portion of this material was given a second treatment with magnesium oxide–Darco as above. It was then dissolved in dimethylformamide, in which it was quite soluble, given a Darco treatment, and reprecipitated by the addition of an equal amount of alcohol. This procedure was repeated twice more, and the product was then treated once more with magnesium oxide–Darco as above. It was obtained as a bright yellow microcrystalline solid which melted with decomposition at 237–239° (immersed at 180°).

Anal. Calcd. for $C_{21}H_{24}N_8O_5 \cdot H_2O$: C, 51.8; H, 5.39; N, 23.0. Found: C, 51.7; H, 5.79; N, 23.4.

In 0.1 *N* sodium hydroxide III showed ultraviolet absorption maxima at 277 and 378 mμ; in 0.1 *N* hydrochloric acid, maxima were at 295 and 344 mμ.

Oxidation of III.—Two-tenths of a gram of III was dissolved in 100 ml. of water by the addition of dilute sodium hydroxide solution to about *p*H 12. It was heated to 90–95° and potassium permanganate was added until the

(16) Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705–710 (1947).

purple color persisted for twenty minutes. A little sodium bisulfite was added to decolorize the solution and it was filtered hot and acidified. After cooling, the precipitate was collected and dissolved in dilute sodium hydroxide to give about 1.2 ml. of solution. The sodium salt was precipitated by the addition of solid sodium hydroxide, collected, and washed with 5 *N* sodium hydroxide. It was then dissolved in 10 ml. of hot water, precipitated with acid, and collected by centrifugation. There was obtained 0.045 g. of 2-amino-4-hydroxypteridine-6-carboxylic acid,[15] identified by comparison of its ultraviolet absorption spectrum with that of an authentic sample.

2,4-Diamino-6-methylaminopyrimidine.—A mixture of 12 g. of 2,4-diamino-6-chloropyrimidine and 15.5 g. of methylamine in 100 ml. of absolute alcohol was heated in an autoclave at 120° for three hours. The resultant solution, which was dark yellow with a blue fluorescence, was filtered from a small amount of insoluble material and vacuum distilled to remove the alcohol. The residual oil was dissolved in 60 ml. of warm water and filtered from a small dark precipitate. The mixture was cooled and 50% sodium hydroxide added until an oil separated, and this was then extracted with isopropyl acetate. On cooling the ester extracts, a yellow solid crystallized; dry weight, 3.4 g. This was recrystallized twice from alcohol, and then melted at 192–194°.

Anal. Calcd. for $C_5H_9N_5$: C, 43.1; H, 6.52; N, 50.4. Found: C, 42.9; H, 6.55; N, 50.7.

2-(or 4)-Amino-4(or 2),6-bismethylaminopyrimidine.—A reaction was carried out exactly similar to the above, except that it was heated at 170° for three hours. The product was worked up in the same fashion. It was isolated from solution in isopropyl acetate by evaporation to an oil which soon solidified for the most part. Upon washing this with cold isopropyl acetate and then recrystallizing from this solvent twice, a white crystalline solid melting between 165–185° was obtained. This was probably a mixture of 2-amino-4,6-bismethylaminopyrimidine and 4-amino-2,6-bismethylaminopyrimidine. It had the following analysis:

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.0; H, 7.24; N, 45.7. Found: C, 46.9; H, 7.43; N, 45.9.

2,4-Diamino-5-nitroso-6-methylaminopyrimidine.—Upon nitrosating 2,4-diamino-6-methylaminopyrimidine by the same procedure as described above, a shiny, cherry-red product was obtained which after reprecipitation from acid solution melted with decomposition at 245–247°.

Anal. Calcd. for $C_5H_8N_6O \cdot {}^1/_2H_2O$: C, 33.9; H, 5.12; N, 47.4. Found: C, 34.0; H, 5.32; N, 47.7.

2,4,5-Triamino-6-methylaminopyrimidine Sulfate.—2,4-Diamino-5-nitroso-6-methylaminopyrimidine was reduced with sodium dithionite in a manner similar to that described for the 6-dimethylamino derivative.

Anal. Calcd. for $C_5H_{10}N_6 \cdot H_2SO_4$: C, 23.8; H, 4.80; N, 33.3; S, 12.7. Found: C, 23.5; H, 4.71; N, 33.7; S, 12.4.

4-Methylaminopteroylglutamic Acid (II).—2,4,5-Triamino-6-methylaminopyrimidine sulfate was condensed with *p*-aminobenzoylglutamic acid and α,β-dibromopropionaldehyde in a fashion similar to the above described reaction with the 6-dimethylamino derivative. From a reaction using 2.7 g. of 2,4,5-triamino-6-methylaminopyrimidine sulfate, 7.2 g. of crude product was obtained having a chemical assay of 10% as 4-methylaminopteroylglutamic acid.

2,4-Diamino-6-(1-piperidyl)-pyrimidine.—Four hundred grams of 2,4-diamino-6-chloropyrimidine was mixed with 1200 ml. of piperidine and slowly heated over a one hour period to 100° on the steam-bath, and then heated at that temperature for an additional two and one-half hours. Three hundred milliliters of alcohol was added to the hot mixture, which was then filtered from piperidine hydrochloride. The precipitate was washed with additional alcohol. The solvents were distilled from the filtrate under vacuum, whereupon the residue crystallized. This was taken up in 2 l. of water and warmed until the solid was partially dissolved and partially converted to an oil, leaving no solid. On cooling and stirring well, the product crystallized. It was filtered off at 10° and dried at 45°, yielding 330 g. (62%) of 2,4-diamino-6-(1-piperidyl)-pyrimidine. A sample was recrystallized for analysis from petroleum ether b. p. 93–162°, yielding silvery white shiny plates melting at 135.5–136.5°.

Anal. Calcd. for $C_9H_{15}N_5$: C, 55.9; H, 7.82; N, 36.2. Found: C, 56.1; H, 7.89; N, 36.4.

2,4-Diamino-5-nitroso-6-(1-piperidyl)-pyrimidine.—The nitrosation of 2,4-diamino-6-(1-piperidyl)-pyrimidine was carried out in the same fashion as that used for the 6-dimethylamino derivative. A bright red nitroso derivative was formed which melted with decomposition at 211.5–213.0°.

Anal. Calcd. for $C_9H_{14}N_6O$: C, 48.6; H, 6.35; N, 37.8. Found: C, 48.6; H, 6.46; N, 37.7.

2,4,5-Triamino-6-(1-piperidyl)-pyrimidine Sulfate.—The reduction of 2,4-diamino-5-nitroso-6-(1-piperidyl)-pyrimidine was carried out as previously described for the dimethylamino derivative. The product in this case was very soluble, which explains a lower yield (60%).

Anal. Calcd. for $C_9H_{16}N_6 \cdot H_2SO_4$: C, 35.3; H, 5.92; N, 27.4; S, 10.5. Found: C, 35.1; H, 5.97; N, 27.7; S, 10.5.

2-Amino-4-(1-piperidyl)-6,7-dimethylpteridine.—Two grams of 2,4,5-triamino-6-(1-piperidyl)-pyrimidine sulfate was dissolved in 30 ml. of water and heated to 80°. A solution of 0.65 g. of diacetyl in 5 ml. of water was added over a five-minute period. The mixture was heated at 80° for one-half hour, and then neutralized with ammonium hydroxide. An orange precipitate formed. This was filtered off after cooling, washed with water, and dried at 50°; weight, 1.1 g. The product was crystallized twice from dimethylformamide and then from 50% alcohol. Cream colored crystals were formed which melted at 214.5–216°.

Anal. Calcd. for $C_{13}H_{18}N_6$: C, 60.5; H, 7.02; N, 32.5. Found: C, 60.6; H, 7.06; N, 32.8.

Ultraviolet absorption maxima were at 234, 271 and 377 mμ in 0.1 *N* sodium hydroxide; at 345 mμ with plateaus at 255 and 305 mμ in 0.1 *N* hydrochloric acid.

4-(1-Piperidyl)-pteroylglutamic Acid (IV).—The condensation of 2,4,5-triamino-6-(piperidyl)-pyrimidine sulfate with *p*-aminobenzoylglutamic acid and α,β-dibromopropionaldehyde was carried out in the same fashion as that described for the 4-dimethylamino derivative. In a run using 230 g. of 2,4,5-triamino-6-(1-piperidyl)-pyrimidine sulfate, 260 g. of golden brown crude product was obtained, which gave a chemical assay indicating the presence of 44 g. of 4-(1-piperidyl)-pteroylglutamic acid. This was purified to a chemical assay of 73.4% by methods analogous to those described for the 4-dimethylamino derivative.

Oxidation of IV.—Oxidation by a procedure similar to that described above for III yielded 2-amino-4-hydroxypteridine-6-carboxylic acid.[15]

4-Dimethylamino-N^{10}-methylpteroylglutamic Acid (V).—This compound was prepared by a process similar to that described for III above, from 2,4,5-triamino-6-dimethylaminopyrimidine sulfate, *p*-methylaminobenzoylglutamic acid,[4b] and 2,3-dibromopropionaldehyde. The crude product (44.7 g.) was added to 3300 ml. of water preheated to 80°. Just enough 5 *N* sodium hydroxide (71 ml.) was added to give solution. After fifteen minutes 10 g. of calcium chloride in 25 ml. of water was added, and the insoluble material was removed by filtration with Hyflo–Supercel. To the filtrate was added 10% zinc chloride solution to reduce the *p*H to 10.8, and the precipitate was filtered off and discarded. The filtrate at 80° was adjusted to *p*H 4, cooled to 5°, and filtered with Hyflo–Supercel. The cake was slurried in 2500 ml. of water at 80°, sodium hydroxide added to *p*H 10.5–11.0, and then while cooling slowly to 20° the solution was gradually adjusted to *p*H 7. The solution was clarified and adjusted to *p*H 4, cooled, and the product collected on the

filter. It was taken up in 2000 ml. of water at 80° with 5 g. of magnesium oxide and 5 g. of Darco G-60, filtered hot, and the filtrate was then neutralized to *p*H 4 and cooled to 5°. The bright yellow product was filtered, washed with water and acetone and dried at 50°. The purity, as estimated from the ultraviolet absorption curve, was about 80%. In 0.1 *N* sodium hydroxide solution it showed maxima at 305 and 378 mμ, a plateau at 278–285 mμ, and a minimum at 252 mμ. In 0.1 *N* hydrochloric acid it showed a maximum at 307 mμ, a plateau at 248–255 mμ, and a shoulder at 340–355 mμ.

Synthesis of Pteroylglutamic Acid by Anaerobic Hydrolysis of 4-Alkylamino Derivatives.—A. From 4-Dimethylaminopteroylglutamic Acid (III).—To 20 ml. of oxygen-free 1 *N* sodium hydroxide was added 0.200 g. of 69.6% 4-dimethylaminopteroylglutamic acid. The solution was heated on the steam-bath for five hours under nitrogen. It was then diluted to 100 ml., and poured into 45 ml. of 30% acetic acid solution. The mixture was cooled and the precipitate collected; dry weight, 0.0977 g. This material had a chemical assay of 69.9%, and a bioassay of 75% as compared to pteroylglutamic acid as a growth-stimulant for *S. faecalis* R.

B. **From 4-(1-Piperidyl)-pteroylglutamic Acid (IV).**—A 0.680-g. sample of 4-(1-piperidyl)-pteroylglutamic acid of 73.4% purity was treated in the same fashion as described above, yielding 0.398 g. of a product with a chemical assay of 63.5%, and a bioassay of 56.5% as growth-stimulant for *S. faecalis* R.

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Summary

A series of 4-alkylaminopteroylglutamic acid derivatives has been synthesized, in which are included 4-dimethylaminopteroylglutamic acid and its 10-methyl-derivative, 4-(1-piperidyl)-pteroylglutamic acid and 4-methylaminopteroylglutamic acid.

Substitution on the 4-amino group of 4-aminopteroylglutamic acid (I), a potent antagonist for pteroylglutamic acid, produced substances of much lower toxicity.

4-Alkylaminopteroylglutamic acids were readily converted to pteroylglutamic acid by heating in alkali under anaerobic conditions.

A number of new 6-alkylaminopyrimidines were prepared as intermediates.

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n-Alkyl Lactates and their Acetates

BY C. E. REHBERG AND MARION B. DIXON

The literature contains many references to various *n*-alkyl lactates and their acetates (*n*-alkyl α-acetoxypropionates), but no comprehensive treatment of the physical constants of these esters as related to molecular structure; nor are adequate data available for such treatment. When the available data are tabulated, many wide discrepancies, as well as numerous blanks, are apparent.

Wood, Such and Scarf[2] prepared the optically active *n*-alkyl L-lactates from the methyl to the nonyl ester. Since they were primarily interested in optical properties, the only other property measured with precision was density. Smith and Claborn[3] reported the boiling point, specific gravity and refractive index for several *n*-alkyl lactates and acetoxypropionates.

In the present work, the lactates and acetoxypropionates of eleven *n*-alkanols having one to sixteen carbon atoms were prepared, and a systematic study of their physical properties was made. The correlation of these properties as linear functions of the number of carbon atoms in the compounds furnishes excellent checks on the purity of these esters and the accuracy of the physical measurements. These correlations are also useful for prediction of the properties of homologs not prepared and of properties at temperatures and pressures other than those studied.

Table I shows the yields and analyses of esters not previously reported. Table II gives physical properties of all the esters studied.

TABLE I

YIELDS AND ANALYSES OF *n*-ALKYL LACTATES AND α-ACETOXYPROPIONATES

Ester	Yield, %	Sapn. equiv. Calcd.	Sapn. equiv. Found	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Decyl lactate	65	230.3	228.9	67.8	67.6	..	..
Tetradecyl lactate	69	286.4	286.8	71.3	70.7	12.0	11.9
Hexadecyl lactate	65	314.5	319.5	72.6	72.4	12.2	12.1
Octyl acetoxypropionate	96	122.2	121.3	63.9	63.9	9.9	10.1
Decyl acetoxypropionate	97	136.2	138.2	66.1	65.8	10.4	10.4
Tetradecyl acetoxypropionate	79	164.2	164.2	69.5	69.4	11.1	11.3
Hexadecyl acetoxypropionate	93	178.3	176.8	70.7	70.8	11.3	11.4

Boiling Points and Vapor Pressures.—The boiling points at various pressures are plotted in

(1) One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

(2) Wood, Such and Scarf, *J. Chem. Soc.*, **123**, 600 (1923).

(3) Smith and Claborn, *Ind. Eng. Chem.*, **32**, 692 (1940).