(~100 mm.), especially with rich mixtures, though this probably only represents more effective quenching of chemi-luminescence. It was also noted that the radiation emitted by mixtures just above the lower limit was more bluish and much less intense. This recalls Frankland's observation[4] that a slow current of boron triethyl (or trimethyl) vapor issuing into air burned with a faint blue flame—"the temperature of which is so low that a finger may be held in it for some time without much inconvenience." Evidently, cool flame phenomena are involved near the limit. It was interesting to find that with the coated reaction bulb this cool flame was no longer observed. The bright green flash was observed right down to the limit which, as already stated, was correspondingly higher.

We have no evidence to present regarding the reaction below the low pressure limit, except that there was no pressure-change or detectable deposit in 1,000 seconds. Experiments of Bamford and Newitt[5] on the oxidation of boron trimethyl and tri-*n*-propyl, by slowly admitting oxygen to the vapor at about 1 mm., indicate a very rapid absorption up to the equivalent of $R_3B:O_2$, with no separation of a condensed product. According to Frankland,[4,6] the slow admission of oxygen or air to liquid boron triethyl produces ethane-boronic diethyl ester, $C_2H_5B(OC_2H_5)$. In any event, there is no evidence of the separation of a solid at these low pressures until the ignition limit is passed.

(4) E. Frankland, *J. Chem. Soc.*, **15**, 363 (1862).

(5) C. H. Bamford and D. M. Newitt, *ibid.*, 695 (1946).

(6) See also Krause, *et al.*, *Ber.*, **61**, 271 (1928); **63**, 934 (1930); a similar observation on boron tri-*n*-butyl by Johnson and Van Campen, THIS JOURNAL, **60**, 121 (1938).

This is in contrast to the oxidation of zinc dimethyl vapor where a white mist begins to form immediately on mixing with oxygen.

Finally, a few experiments on the induced ignition of *n*-butane by means of boron tri-ethyl have been carried out, by admitting a stoichiometric mixture of oxygen and *n*-butane (13.3 vol. %) to a bulb containing boron triethyl vapor, the total pressure being 100 mm. With 1 mm. of boron triethyl there was no reaction, but with 3 mm. of boron triethyl there was a violent explosion which travelled back through 6 mm. i. d. tubing to the reservoir flask. However, with 5 or 10 mm. of boron triethyl there was only a faint flash, which was much weaker than that in absence of *n*-butane, and a negligible pressure change.[7] There thus appear to be sharp pressure limits to this induced oxidation.

Summary

1. Boron triethyl vapor ignites spontaneously in oxygen at partial pressures below 1 mm. in a Pyrex bulb held at 0°.

2. The product of minimum pressure and bulb diameter is constant, indicating that chains start in the gas phase and end on the wall. When the glass surface is coated with reaction products, the minimum pressure is greater.

3. There is evidence of a cool flame phenomenon.

4. Boron triethyl will ignite a *n*-butane–oxygen mixture within narrow pressure limits.

(7) The 3 mm. mixture produced a large pressure decrease, presumably due to condensation of water vapor formed by the combustion.

PRINCETON, NEW JERSEY RECEIVED OCTOBER 18, 1947

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

Analogs of Pteroylglutamic Acid. I. N^{10}-Alkylpteroic Acid and Derivatives

BY DONNA B. COSULICH AND JAMES M. SMITH, JR.

The structure of pteroylglutamic acid[1] has been demonstrated and several methods of synthesis have been described.[1,2]

This factor of the vitamin B complex is identical with the liver *L. casei* factor[3] and apparently is related to a number of other substances[4] isolated from natural sources. All are necessary for the normal growth and development of certain animals and microörganisms. The question immediately arises as to what the biological effect will be when variations are introduced into the structure of the vitamin by chemical methods of synthesis. There is always the possibility of enhancing the desirable effects of the vitamin, and also of producing new compounds which can be used in treating other syndromes. The use of pteroylglutamic acid antagonists in the treatment of blood dyscrasias, leukemia for example, has been suggested by Franklin, Stokstad, Belt and Jukes.[5a]

Variations of the structure and study of the relationship between structure and biological activity have already received the attention of workers in the field. Dibromobutyraldehyde has

(1) (a) Angier, *et al.*, *Science*, **103**, 667 (1946); (b) Mowat, *et al.*, THIS JOURNAL, **70**, 14 (1948).

(2) (a) Waller, *et al.*, *ibid.*, **70**, 19 (1948); (b) Hultquist, *et al.*, *ibid.*, **70**, 23 (1948); (c) Angier, *et al.*, *ibid.*, **70**, 25 (1948); (d) Boothe, *et al.*, *ibid.*, **70**, 27 (1948).

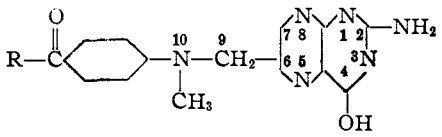
(3) Stokstad, Hutchings and SubbaRow, *ibid.*, **70**, 3 (1948).

(4) (a) Hutchings, Stokstad, Bohonos, Sloane and SubbaRow, *ibid.*, **70**, 1 (1948); (b) Snell and Peterson, *J. Bact.*, **39**, 273 (1940); (c) Hutchings, Bohonos and Peterson, *J. Biol. Chem.*, **141**, 521 (1941); (d) Mitchell, Snell and Williams, THIS JOURNAL, **63**, 2284 (1941); (e) Pfiffner, *et al.*, *Science*, **97**, 404 (1943).

(5) (a) Franklin, Stokstad, Belt and Jukes, *J. Biol. Chem.*, **169**, 427 (1947); (b) Martin, Tolman and Moss, *Archives of Biochemistry*, **12**, 318 (1947); *Science*, **106**, 168 (1947); (c) Franklin, Stokstad and Jukes, *Proc. Soc. Exptl. Biol. Med.*, **65**, 368 (1947); (d) Welch, Heinle, Sharpe, George and Epstein, *ibid.*, **65**, 364 (1947).

been substituted for dibromopropionaldehyde in the synthesis of pteroylglutamic acid[2a] to give a product which is an antagonist for pteroylglutamic acid.[5] The proof of structure of the active material has not been reported, but the crude has been designated as "7-methylfolic acid."[5b] Pteroylaspartic acid has been synthesized in pure form and shows pteroylglutamic acid antagonist activity on a number of species.[6] The derivative of pteroylglutamic acid in which the 4-hydroxyl group is replaced by an amino group has been reported by Seeger, Smith and Hultquist.[7a] It is a powerful antagonist for pteroylglutamic acid. 2,4-Diaminopteridines having antagonist action have been investigated by other workers.[7b]

The present paper describes the synthesis of a series of N^{10}-substituted derivatives of pteroylglutamic acid which are antagonists, as for example N-[4-{N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid, (I) hereafter designated as N^{10}-methylpteroylglutamic acid.



I, R is $HOOCCH_2CH_2CH(COOH)NH-$
II, R is HO—

N^{10}-Methylpteroic acid (II) is the simplest member of the series, and also one of the most active antagonists. It was prepared from dibromopropionaldehyde, 2,4,5-triamino-6-hydroxypyrimidine, and *p*-methylaminobenzoic acid, and purified by the methods described for the synthesis and purification of pteroylglutamic acid.[2a] The other compounds reported in this series were all prepared in a similar manner, only those showing significant activity in the crude being purified.

Alkaline permanganate oxidation of N^{10}-methylpteroic acid yielded 2-amino-4-hydroxypteridine-6-carboxylic acid, which was identical with that obtained by the alkaline aerobic oxidation of pteroylglutamic and pteroic acids.[1a] This shows that the point of attachment of the side chain is the 6-position. However, alkaline aerobic oxidation of N^{10}-methylpteroic acid under conditions which cleaved pteroylglutamic and pteroic acids yielded only the unchanged material.

It was necessary to obtain pure secondary amines to preclude the presence of pteroic and pteroylglutamic acids which would mask the growth inhibiting properties of the N^{10}-alkylated derivatives. As a consequence, useful methods for the preparation of pure monoalkylated *p*-aminobenzoic and *p*-aminobenzoylglutamic acids were devised. These included the treatment of the corresponding iodo compounds with alkyl amines, methylation of aminobenzoic acid by the zinc–alkali reduction of the amino acid and formaldehyde, and the reaction of alkyl iodides with sodium ethyl *p*-formamidobenzoate followed by hydrolysis of the formyl group.

The biological properties of the N^{10}-alkylpteroic acid derivatives have been examined by Dr. E. L. R. Stokstad and Dr. B. L. Hutchings of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. For N^{10}-methylpteroylglutamic acid the inhibition ratio for half-maximum inhibition of the growth of *Streptococcus faecalis R* was 2.0 at a concentration of pteroylglutamic acid of 0.1 microgram per 10 ml. See also Table I.

The details of the biological work will be published elsewhere.

Experimental[8,9]

***p*-Methylaminobenzoic Acid.**[10]—Sixty grams of *p*-aminobenzoic acid was dissolved in 90 ml. of water and 17.5 g. of sodium hydroxide. The resulting solution, and 66 g. of 40% formaldehyde solution, were added simultaneously over a two to three-hour period to a slurry of 90 g. of zinc dust in 205 g. of 50% sodium hydroxide solution and 100 ml. of water. The temperature was maintained at 90–95°. Heating at this temperature was continued while 40 g. more of 40% formaldehyde was added. The total heating time was five to seven hours. Excess zinc was removed by filtration, and the filtrate was allowed to stand several days. The sodium salt of *p*-methylaminobenzoic acid crystallized out. It was filtered off, dissolved in water, and the acid precipitated by adjusting the solution to *p*H 3. This was essentially pure *p*-methylaminobenzoic acid; m. p. 158–161.5°. A mixture melting point with a sample prepared by the method of Houben and Schottmüller[10] gave no depression.

N-Methylanthranilic acid[11] was prepared in a similar experiment.

N^{10}-Methylpteroic Acid.—This compound was synthesized by the method of Waller, *et al.*,[2a] except that *p*-methylaminobenzoic acid was substituted for *p*-aminobenzoylglutamic acid. A mixture of 12.6 g. of crude N^{10}-methylpteroic acid, 9 g. of lime, and 1750 ml. of water, was heated at 60° for forty minutes. After the addition of 25 g. of Hyflo-Supercel the mixture was filtered and the cake washed with 750 ml. of water at 60°. The filtrate and washing were adjusted to *p*H 3 with hydrochloric acid and cooled to 20°. After the addition of 25 g. of Hyflo-Supercel, the mixture was filtered. The cake was washed with water and then slurried in 1 liter of water and sodium hydroxide was added to obtain *p*H 11–12. After heating at 80° for ten minutes, the *p*H of the solution was adjusted to 7, and the mixture then cooled to 20° and filtered. The filtrate was treated with hydrochloric acid to *p*H 3–4. The precipitated material was separated by filtration with Hyflo, slurried in water to give 0.75 g./liter concentration and enough magnesium oxide to obtain about *p*H 9 at 80°, and filtered hot with 0.5 g. of charcoal. The filtrate at 80° was adjusted to *p*H 3–4 with hydrochloric acid and cooled to 20°. Yellow rosets of N^{10}-methylpteroic acid crystallized out and were purified for analysis by dissolving (at 0.25 g./l.) in hot dilute sodium hydroxide (*p*H 8–9), clarifying with Hyflo filter-aid, and adjusting the filtrate to *p*H 3–4 while at 90°. On cooling analytically pure yellow microcrystalline material was isolated which had an ultraviolet absorption curve quite similar to that predicted from consideration of data on the ultraviolet curves of pteroic acid, *p*-methylaminobenzoic acid, and *p*-dimethylaminobenzoic acid. See Table I and Fig. 1 for

(6) Hutchings, *et al.*, *J. Biol. Chem.*, **170**, 323 (1947).

(7) (a) Seeger, Smith and Hultquist, THIS JOURNAL, **69**, 2567 (1947). (b) Mallette, Taylor and Cain, *ibid.*, **69**, 1814 (1947).

(8) All melting points are corrected.

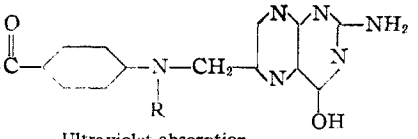
(9) Microanalyses were done by Mr. O. Sundberg and assistants.

(10) Houben and Schottmüller, *Ber.*, **42**, 3739 (1909).

(11) Houben and Brassert, *ibid.*, **39**, 3234 (1906).

TABLE I

N^{10}-SUBSTITUTED PTEROIC AND PTEROYLGLUTAMIC ACIDS



R	R_1	Purity	Ultraviolet absorption spectra maxima, λ mμ.[a] in 0.1 N NaOH			in 0.1 N HCl	Antagonist activity[b]
$-CH_3$	—OH	Analytical	255	290	366	313	15.00
$-CH_3$	—OH	Crude	...	...	...	...	2.00
$-C_2H_5$	—OH	91.2%[c]	254	297	366	314	1.076
$-C_2H_5$	—OH	Crude	...	...	...	...	0.075
$-C_4H_9$	—OH	85%[c]	255	298	367	315	0.135
$-C_4H_9$	—OH	Crude	...	...	...	...	.....
$-CH_2COOH$	—OH	Crude	...	...	...	...	0.022
$-CH_2C_6H_5$	—OH	Crude	...	...	...	...	.....
$-CH_2COC_6H_5$	—OH	Crude	...	...	...	...	0.01
$-CH_3$	—l(+)glutamic acid	Analytical	255	302	368	307	100.00
		Crude	...	...	...	...	22.50
$-CH_2COC_6H_5$	—l(+)glutamic acid	Crude	...	...	...	...	0.01

[a] See footnote to Fig. 1. [b] An arbitrary value of 100 is assigned for the antagonist activity of N^{10}-methylpteroylglutamic acid, for half-maximum inhibition of the growth of *Streptococcus faecalis R*. Values for other compounds are reported in terms of the standard. [c] Estimated by ultraviolet absorption.

biological and ultraviolet absorption data. The analytical sample was dried at 100° and 1 mm. for seven to eight hours.

Anal. Calcd. for $C_{15}H_{14}N_6O_3$: C, 55.2; H, 4.29; N, 25.75. Found (corrected for 2.71% ash): C, 55.3; H, 4.56; N, 25.4.

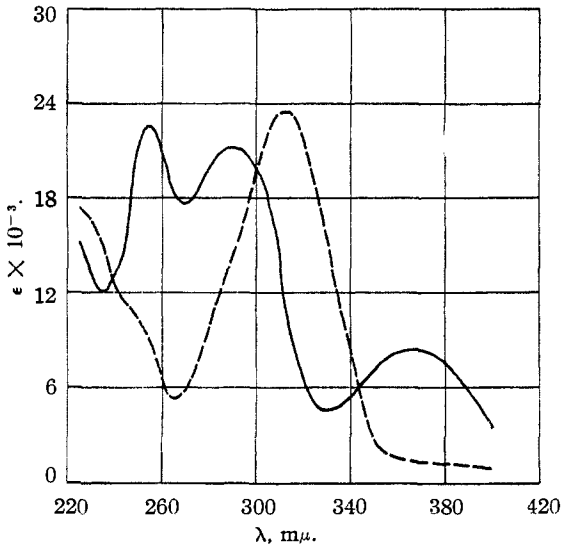


Fig. 1.—Ultraviolet absorption spectra[a] of N^{10}-methylpteroic acid: ——— in 0.1 *N* sodium hydroxide; ---------- in 0.1 *N* hydrochloric acid.

Permanganate Oxidation of N^{10}-Methylpteroic Acid.—A hot solution of 730 mg. of N^{10}-methylpteroic acid in 250 ml. of 1 *N* sodium hydroxide was treated with 2% potassium permanganate solution until the solution maintained a dark green color. After adding sodium sulfite to destroy this color, manganese dioxide was filtered off and the filtrate adjusted to *p*H 3–4 with dilute hydrochloric acid. The mixture was cooled, and centrifuged. The crude moist product was purified by adding a few drops of 5 *N* sodium hydroxide to dissolve, and then 4.4 g. of sodium hydroxide pellets to the 22 ml. of solution to give a 5 *N* caustic solution. On cooling, the disodium salt separated out, was filtered off, and precipitated as the free acid by dissolving in water and adjusting to *p*H 3–4. The disodium salt was isolated a second time to give material which was shown to be 2-amino-4-hydroxypteridine-6-carboxylic acid by comparison of the ultraviolet absorption curve with that of an authentic sample.

Attempted Alkaline Aerobic Oxidation of N^{10}-Methylpteroic Acid.—A solution of 500 mg. of N^{10}-methylpteroic acid in 25 ml. of *N* sodium hydroxide was heated at 100°

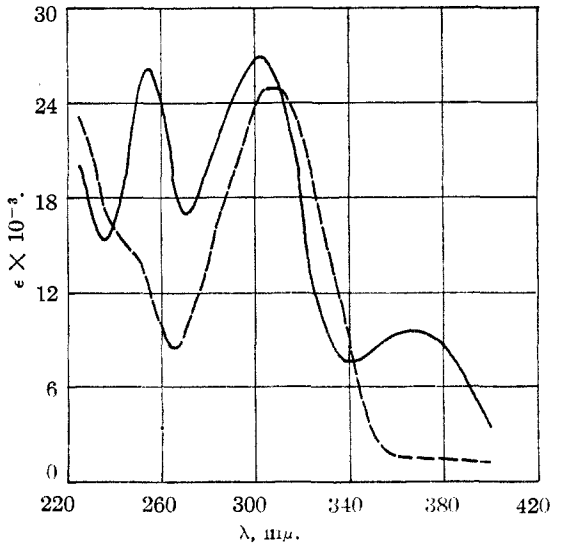


Fig. 2.—Ultraviolet absorption spectra[a] of N^{10}-methylpteroylglutamic acid monohydrate: ——— in 0.1 *N* sodium hydroxide; ---------- in 0.1 *N* hydrochloric acid.

[a] *E* is the molecular extinction coefficient as defined by $I = I_0 10^{-Ecl}$ 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. cells at 5mμ 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

for six hours with a rapid stream of oxygen bubbling through. After cooling and clarifying with 0.5 g. of charcoal, the filtrate was adjusted to 5 *N* by adding 4 g. of sodium hydroxide pellets. When cooled in the icebox overnight, yellow crystals separated, which were filtered and dissolved in water. The yellow solution, after clarifying with 0.2 g. of charcoal, was adjusted to *p*H 3–4 with hydrochloric acid, whereupon an orange substance precipitated. This was isolated by centrifugation and shown to be unchanged N^{10}-methylpteroic acid by comparison of its ultraviolet absorption curve with an authentic sample.

Sodium Ethyl *p*-Formamidobenzoate.—A solution of 82.54 g. of ethyl *p*-aminobenzoate in 578 ml. of ether was treated with 12.5 g. of sodium, added in small pieces. To this was added slowly 37 g. of ethyl formate and the mixture was allowed to stand overnight. The product, isolated as a fine yellow powder, had a neutralization equivalent of 235.4 (theoretical for $C_{10}H_{10}NO_3Na$, 215). A sample was converted to ethyl *p*-formamidobenzoate by neutralization of an aqueous solution with acetic acid; the melting point, 146–149°, agreed with the literature value.[12]

***p*-Ethylaminobenzoic Acid.**[13]—A mixture of 21.5 g. of sodium ethyl *p*-formamidobenzoate, 15.6 g. of ethyl iodide, 125 ml. of ethyl alcohol and 13.4 g. of potassium hydroxide (86%), was refluxed for one hour. Then 25 ml. of water was added and the solution was poured into cold water and neutralized with acetic acid. The crude material was easily purified by two reprecipitations from sodium hydroxide solution to give *p*-ethylaminobenzoic acid. The melting point, 177.9–178.9°, agreed with that obtained by the method of Houben and Freund.[13]

p-Ethylaminobenzoic acid was also prepared from *p*-iodobenzoic acid[14] and ethylamine in a sealed tube in a manner analogous to that described below for the preparation of *p*-methylaminobenzoylglutamic acid.

N^{10}-Ethylpteroic Acid.—This compound was prepared according to the procedure described by Waller, *et al.*,[2a] except that *p*-ethylaminobenzoic acid was substituted for *p*-aminobenzoylglutamic acid. It was purified by a process similar to that described above for N^{10}-methylpteroic acid (see Table I).

N^{10}-Butylpteroic Acid.—This was prepared from *p*-butylaminobenzoic acid[15] and purified as indicated for the N^{10}-ethyl compound above (see Table I).

Ethyl N-(4-Carbethoxyphenyl)-glycinate.—A mixture of 12.25 g. of ethyl chloroacetate and 33 g. of ethyl *p*-aminobenzoate was heated at 130–140° for five to six hours. It was cooled, slurried in ether and filtered. The filtrate was freed of ether by evaporation and the residue was distilled at 215° at 8–11 mm. pressure. The distillate was dissolved in ether and extracted with a small amount of 0.5 *N* hydrochloric acid. Evaporation of the washed and dried ether layer gave the ester which was purified further by recrystallization from aqueous alcohol; m. p. 62.5–62.9°.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.1; H, 6.77; N, 5.8. Found: C, 62.1; H, 6.87; N, 5.73.

This compound is soluble in acetone, ether, alcohol, isopropyl acetate, carbon tetrachloride and petroleum ether; insoluble in water and carbon disulfide.

N-(4-Carboxyphenyl)-glycine.—The ester above (8.8 g.) was boiled three to four hours in 5 *N* sodium hydroxide solution. On diluting, cooling and acidifying, the pure acid separated out; m. p. 245.7–247.1°, neut. equiv. 98.8 (the theoretical value is 97.5 for $C_9H_9O_4N$).

N^{10}-Carboxymethylpteroic Acid.—This was prepared as a crude as described for other N^{10}-pteroic acids, from N-(4-carboxyphenyl)-glycine.

Other N^{10} Derivatives of Pteroic Acid.—In similar experiments N^{10}-phenacylpteroic acid and N^{10}-benzylpteroic acid were prepared as crudes from *p*-phenacylaminobenzoic acid[16] and *p*-benzylaminobenzoic acid.[1b]

***p*-Iodobenzoylglutamic Acid.**—A solution of 26.5 g. of *p*-aminobenzoylglutamic acid in 50 g. of 35% hydrochloric acid and 150 ml. of water was cooled to 5–10° and 8.5 g. of sodium nitrite in 40 ml. of water was added, until the solution gave a blue spot on starch–iodide test paper. Then 25 g. of potassium iodide in 50 ml. of water was added and the mixture was allowed to stand overnight. It was warmed to 50°, cooled, washed by decantation, and filtered. The crude product was purified by recrystallization from dilute alcohol; m. p. 173.7–176.1°; $[\alpha]^{26}D$ + 16.32 (1 *N* sodium hydroxide).

Anal. Calcd. for $C_{12}H_{13}NO_5I$: C, 38.1; H, 3.44; N, 3.7; I, 33.6. Found: C, 37.7; H, 3.21; N, 3.93; I, 33.6.

***p*-Methylaminobenzoylglutamic Acid.**—A solution of 7.6 g. of *p*-iodobenzoylglutamic acid in 8 ml. of water and sodium hydroxide to give *p*H 8–9 was treated with 8 ml. of aqueous methylamine (24.15 g./100 ml.) and 0.02 g. of fine copper powder in a sealed tube at 125° for three hours. The insolubles were removed by filtration and the excess methylamine was removed by evaporation under vacuum. The sirupy residue was diluted with alcohol to give the disodium *p*-methylaminobenzoylglutamate. The free acid was obtained as an oil by acidification of an aqueous solution of the sodium salt.

Diethyl *p*-Methylaminobenzoylglutamate.—The diethyl ester was obtained by dissolving 3.5 g. of the disodium salt above in 60 ml. of alcoholic hydrogen chloride (20 g./100 ml.) and allowing it to stand three days. After diluting with water and clarifying, the ester was precipitated by the addition of ammonium hydroxide to *p*H 7–8. The crude ester was purified by recrystallization from dilute alcohol; m. p. 89.8–91.0°; $[\alpha]^{25}D$ −21° (1 *N* HCl).

Anal. Calcd. for $C_{17}H_{24}N_2O_5$: C, 60.7; H, 7.14; N, 8.33. Found: C, 60.3; H, 7.09; N, 8.48.

N^{10}-Methylpteroylglutamic Acid.—Synthesis was by the method of Waller, *et al.*[2a] The purification was accomplished as indicated above. The pure material was obtained as yellow spherulites (see Table I). The material, dried at 100° and 1 mm. for eight hours, was a monohydrate.

Anal. Calcd. for $C_{20}H_{21}N_7O_6 \cdot H_2O$: C, 50.8; H, 4.86; N, 20.55. Found: C, 50.8; H, 5.06; N, 20.6.

***p*-Phenacylaminobenzoylglutamic Acid.**—*p*-Aminobenzoylglutamic acid (133 g.) was dissolved in water (500 ml.) by the addition of sodium carbonate (60 g.). The solution was heated to 85° and 75 g. of phenacyl chloride was added in three portions of 25 g. of each fifteen minutes apart. Sodium carbonate was added as necessary to keep the solution slightly alkaline. Heating was continued at 90–95° for three hours. On acidification of the cooled solution the product precipitated out. It was purified by recrystallization from alcohol. The yield was 32 g. of material; m.p. 100–104°.

Anal. Calcd. for $C_{20}H_{20}N_2O_6$: C, 62.5; H, 5.21; N, 7.3. Found (corrected for 4.2% ash): C, 63.5; H, 5.85; N, 7.35.

N^{10}-Phenacylpteroylglutamic Acid.—This compound was prepared in crude form by the method of Waller, *et al.*[2b] See Table I.

Acknowledgment.—We are indebted to Mr. Richard L. Shepard for technical assistance in the preparation of certain of these compounds, and to Miss Ruth Abbott for the ultraviolet absorption data.

Summary

1. N-[4-{N-[(2-Amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoyl]-glutamic acid and 4-{N-[(2-amino-4-hydroxy-6-pteridyl)-methyl]-N-methylamino}-benzoic acid, called herein

(12) Cairncross and Bogert, *Coll. Czechoslov. Chem. Communications*, **8**, 63 (1936).

(13) Houben and Freund, *Ber.*, **42**, 4822 (1909).

(14) Willgerodt, *ibid.*, **27**, 2331 (1894).

(15) Fel'dman and Kopeliovich, *J. Applied Chem. (U.S.S.R.)*, **17**, 588 (1944).

(16) Scholtz, *Ber.*, **51**, 1653 (1918).

N^{10}-methylpteroylglutamic acid and N^{10}-methylpteroic acid, have been synthesized in pure crystalline form, and found to be antagonists for pteroylglutamic acid.

2. The purified N^{10}-ethyl- and butyl-, and crude N^{10}-carboxymethyl-, benzyl-, and phenacylpteroic acids and N^{10}-phenacylpteroylglutamic acid have also been prepared. These have a lower order of antagonist activity.

3. Convenient methods of obtaining pure N-monosubstituted aminobenzoic and aminobenzoylglutamic acids have been devised.

BOUND BROOK, NEW JERSEY
RECEIVED DECEMBER 5, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

The Synthesis of Thymine Nucleosides[1,2]

BY DONALD W. VISSER,[3] IRVING GOODMAN AND KARL DITTMER

A method for the synthesis of pyrimidine nucleosides was described by Hilbert and Johnson[4] who prepared 1-glucosyluracil by the reaction of 2,4-diethoxypyrimidine with acetobromoglucose followed by hydrolysis. By this method these and other investigators prepared the uracil nucleosides of D-ribose,[5] L-arabinose,[6] D-xylose,[6] D-glucose[4] and D-galactose.[6] However, the literature contains no conclusive report of the synthesis of thymine nucleosides. The syntheses of the D-ribose, D- and L-arabinose, D-glucose and D-galactose nucleosides of thymine by a modification of this procedure are reported in this paper.

When Schmidt–Nickles and Johnson[7] treated 2,4-diethoxy-5-methylpyrimidine with D-acetobromoglucose at 50° for seven days, they obtained a small amount of a crystalline substance which melted at 316°. We repeated this reaction under similar conditions and isolated a small amount of material which, when crystallized from water, melted at 326° and proved to be thymine. Since no other product was isolated from this reaction mixture, various modifications of the original procedure were studied.

It seemed desirable to provide conditions which would enhance the removal of ethyl bromide, a by-product, which might enter into undesirable side reactions.[7] For this reason the reaction between the acetobromoglucose and 2,4-diethoxy-5-methylpyrimidine was carried out at a pressure of 2 mm. for four days at 50°. Since ethyl bromide was collected in a Dry Ice trap, it was assumed that the desired product was formed, even though it could not be isolated. The complete reaction mixture was then hydrolyzed with dry hydrogen chloride in absolute methanol. After the solvents were removed *in vacuo*, glucosylthymine was crystallized from absolute ethyl alcohol.

In a similar manner the other thymine nucleosides were formed, although each behaved differently. The acetoarabinosylethoxythymine crystallized directly in the reaction mixture. Ribosylthymine did not crystallize upon hydrolysis of the reaction mixture. Therefore, an impure intermediate was isolated by fractional precipitation at low temperatures before hydrolysis. Hydrolysis of this intermediate yielded ribosylthymine.

Experimental

2,4-Diethoxy-5-methylpyrimidine.—The 2,4-diethoxy-5-methylpyrimidine was prepared from thymine (commercial source) according to the directions given by Schmidt–Nickles and Johnson.[7]

D-Acetobromoribose.—The directions of Levene and Tipson[8] were followed for the preparation of D-acetobromoribose except that petroleum ether was not used to facilitate crystallization of the product. The ether solution of the acetobromoribose, after treatment with Norite, was slowly concentrated *in vacuo* to about one-third of its original volume. The large colorless crystals were washed with a small amount of cold, dry ether, and dried over phosphorus pentoxide in a vacuum desiccator. Contact with moisture was avoided throughout the procedure. If the proper precautions were not taken, the acetobromoribose began to decompose within ten to fifteen minutes. Unless it was redissolved in dry ether, treated with Norite and recrystallized, complete decomposition took place within a few hours.

D-Acetobromoarabinose.—This compound was prepared according to the directions of Anderson and Snell.[9]

D-Acetobromoglucose.—D-Acetobromoglucose was prepared by a method similar to that described by Karjala and Link.[10]

D-Acetobromogalactose.—A modification of the method of Levene and Raymond[11] was used for the synthesis of D-acetobromogalactose. One hundred grams of glacial acetic acid was saturated with dry hydrogen bromide and cooled to 0°. To this solution 25 g. of dry, finely-powdered D-galactosepentaacetate was added and anhydrous hydrogen bromide was passed through the suspension at 0–10° with stirring until all the galactosepentaacetate had dissolved. The flask was loosely stoppered and allowed to stand for one hour at room temperature. The hydrogen bromide was removed under vacuum, and the product isolated as described for the preparation of acetobromoribose.

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(9) Anderson and Snell, "Organic Syntheses," Vol. VIII, John Wiley and Sons, New York, N. Y., 1926, p. 18.

(10) Karjala and Link, THIS JOURNAL, **62**, 917 (1940).

(11) Levene and Raymond, *J. Biol. Chem.*, **90**, 247 (1931).